Dear all,

We are happy to welcome you all to Uppsala and the XVth International Symposium on amyloidosis.

Uppsala harbors the oldest University in Scandinavia, founded 1477 and is the home town of Linné (Carolus Linneaus) (1707-1778), the great Swedish botanist, who created a botanical garden, where we meet at the welcome reception.

The large number of high quality abstracts indicates that amyloid diseases are in focus of attention. We are happy to welcome delegates from all over the world, and especially a large number of young scientists, who ensure a continuous development and expansion of research in the field of amyloidosis.

We are also happy to acknowledge the generous support from our sponsors and also from the International Society of Amyloidosis, who supported young investigators participating in the meeting.

This year five debate sessions covering the subjects Pathways for ATTR amyloid formation, Stem cell transplantation or chemotherapy for frontline treatment of AL amyloidosis, Suitable methodology for treatment evaluation, Animal models, and ThT-assay pitfalls can the method be standardized or are there new alternatives? are on the program and we hope these sessions will point to the complexity but facilitate in future studies.

The development of treatment modalities increases the interest and importance of amyloidosis, and this is reflected by the increasing number of meetings dedicated to the disease. Considering this competition, we are very happy that we can welcome a large number of delegates, and look forward to a successful meeting that hopefully will give us all new ideas and inspiration for new projects, as well as new friends and collaborators.

This time of the year is the best in Sweden and Uppsala. The long days and the statistically good chances for sunny days should ensure that you will have a good stay in Uppsala.

Best wishes, and thank you for attending our symposium

Gunilla Westermark (President of the Symposium), Ole B Suhr and Per Westermark
ACKNOWLEDGEMENTS

The organizing committee of the XVth International Symposium on Amyloidosis is grateful to the following sponsors whose generous contributions made the Symposium possible.

**PLATINUM SPONSOR**
- Alnylam
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**GOLD SPONSOR**
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- Bellus Health
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- Ionis Pharmaceuticals

**ADDITIONAL SPONSORS**
- Amyloidosis Research Consortium
- Ebba Biotech
- Taylor & Francis
- The Binding Site Group
## INTERNATIONAL SOCIETY OF AMYLOIDOSIS BOARD

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<td>Bouke P.C. Hazenberg</td>
<td>President</td>
<td>Groningen, Netherlands</td>
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<tr>
<td>Merrill D. Benson</td>
<td>Immediate past President</td>
<td>Indianapolis, USA</td>
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<td>Angela Dispenzieri</td>
<td>Vice president</td>
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<tr>
<td>Lawreen H. Connors</td>
<td>Secretary</td>
<td>Boston, USA</td>
</tr>
<tr>
<td>Steven Zeldenrust</td>
<td>Treasurer</td>
<td>Rochester, USA</td>
</tr>
<tr>
<td>Per Westermark</td>
<td>Editor-in Chief of Amyloid</td>
<td>Uppsala, Sweden</td>
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<tr>
<td>Yukio Ando</td>
<td>Member-at-Large</td>
<td>Kumamoto, Japan</td>
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<td>Vaishali Sanchorawala</td>
<td>Member-at-Large</td>
<td>Boston, USA</td>
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<td>Gunilla T. Westermark</td>
<td>Member-at-Large</td>
<td>Uppsala, Sweden</td>
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ADVISOR BOARD

David Adams, Paris, France
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ORAL PRESENTATIONS:

- The time for oral presentations is limited, consult the program for information. A 15 minutes session includes 8 minutes talk and 5 minutes for questions.

- Presentation should be in PowerPoint and stored on a USB flash drive.

- Speakers should bring their presentations to the session hall at least 1 hour ahead. A media tech will be available for help and provide additional instructions. If you present during the morning session, hand in your presentation the day before.

- Please include the Abstract number and presenter’s name in the PowerPoint file name.

- The data will be handled responsibly and deleted after the presentation.

- The speaker of the oral presentation must be registered for the Symposium.

POSTER PRESENTATIONS:

- Posters are identified by PA (Monday), PB (Tuesday) and PC (Wednesday) followed by the number of the board.

- The size of the poster board is 140 cm high and 100 cm wide. Posters must fit into this format.

- Posters should be mounted preferable before the meeting starts in the morning.

- The presenter should be near his/her poster during allocated poster time.

- The presenter of the poster must be registered for the symposium.

- Posters must be removed at the end of the last poster session of each day.
GENERAL INFORMATION

CONFERENCE VENUE
The conference takes place at Uppsala Konsert & Kongress, Vaksalatorg 1
Phone: +46 18 727 90 20

CONFERENCE SECRETARIAT OPENING HOURS
The secretariat will open on Sunday, July 3 at 13.00 and will be open throughout the conference.

NAME BADGE
Your name badge is your admission to the scientific sessions as well as to coffee and lunches. It should be worn at all times at the conference venue.

INTERNET ACCESS
Wireless Internet access is available at the venue.

Network: UKK
Password: Uppsala1

COFFEE
Coffee will be served in the foyers, level 6 of Uppsala Konsert & Kongress. Please see the program for time and place. You will need your name badge as a ticket.

LUNCH
Lunches will be served in the foyers, level 6 of Uppsala Konsert & Kongress. If you have any dietary requests that you have informed the organisers about in your registration, your name badge has been marked accordingly. Please contact the catering staff.

EXHIBITION AREA
The exhibition opens on Sunday, July 3rd at 13:00. Daily exhibition as stated in the programme. The exhibition will take place on the 6th floor.

SOCIAL EVENTS
Welcome reception, Sunday July 3rd at 19:00 in the Botanical Garden. Address: Villavägen 8. The Botanical Garden is one of Uppsala’s most popular destinations. Here, you can enjoy the grandiose Baroque Garden and visit the 200 year old Orangery with its beautiful grand halls.

If you have registered for the welcome reception you have received two tickets for a beverage (beer, wine or non-alcoholic). You can buy additional beverages in the bar.

Conference Dinner, Wednesday 6 July at 19:00 at Uppsala castle. Address: Rikssalen, Uppsala Slott. Building started on Uppsala Slott in 1549 during the reign of Swedish King Gustav Vasa who intended it as a fortress. Look up from almost any location in Uppsala and you’ll see it on the skyline at Kasåsen. The castle is the location of several major events in the history of Uppsala and Sweden. The castle was also the administrative centre of Uppland for many years, and is today the residence of the County Governor of Uppsala County.

If you have registered for the banquet you have a “D” printed on your name badge. Please bring your name badge as dinner ticket.
OTHER PRACTICAL INFORMATION

MONEY EXCHANGE, CURRENCY
Swedish Krona (SEK) is the official currency in Sweden. There are plenty of cash dispensers in Uppsala. Major international credit cards are accepted in most hotels, shops and restaurants.

SHOPPING IN UPPSALA
Most stores in Uppsala are open 10:00-19:00 on weekdays and 10:00-17:00 on Saturdays. Some stores are open on Sundays as well. Grocery stores usually have longer opening hours.

TIPPING
Service is included in the restaurant bills. Tips, however, are given to show appreciation of a good meal or a special service.

TRANSPORT TO STOCKHOLM ARLANDA INTERNATIONAL AIRPORT
Taxi
You can pre-book a taxi at Taxi Kurir: (+46) 123456 or at www.taxikurir.se or at Uppsala Taxi: +46 18 100 000, www.uppsalataxi.se. The price to get to Stockholm Arlanda International Airport is about SEK 520 (56 Euro).

Bus
Bus 801 runs between Uppsala Central Station and Arlanda once-twice an hour the whole day and parts of the night. The journey takes about 45 minutes and costs 81 SEK (9 Euro). You can buy a ticket at Uppsala Central station. For further information visit: www.ul.se. You can by the ticket with credit card on the bus. It is not possible to pay with cash.

Train
Trains leave Uppsala Central Station for Arlanda Airport 1-3 times/hour from 5:00 until 23:00. The journey takes 15-20 minutes and costs 166 SEK (18 Euro) if purchased in advance at Arlanda train station or Uppsala Central Station.

TOURIST INFORMATION
www.destinationuppsala.se

Uppsala Tourist Center
Kungsgatan 59, 753 21 Uppsala
Tel +46 18 727 48 00

EMERGENCY CALLS
You should call 112 if anything happens which means that an ambulance, the police or the fire brigade needs to be called out. 112 is a special emergency number you can call wherever you may find yourself, from a fixed or a mobile telephone.
INTERNATIONAL CALLS
Dial 00 + country code + area code + phone number. For example to Spain 0034, to Norway 0047.

ELECTRICITY
In Sweden the electrical voltage used is 220/230V.

PHARMACY
There are several pharmacies in Uppsala. Look for ‘Apotek’.

MEDICAL SERVICES
Uppsala University Hospital, Akademiska sjukhuset, is located in central Uppsala. Telephone: +46 18 611 0000. The emergency room is called “Akuten” in Swedish.

SMOKING
Smoking is not allowed in the conference venues, or in any other public indoor establishments such as restaurants, bars, etc.

ORGANIZATION SUPPORT
Academic Conferences – SLU and Uppsala University in cooperation

Office contact details during office hours (8:00-16:00 local time)
Tel: +46 (0)18 67 10 03
E-mail: isa2016@akademikonferens.uu.se

FORCE MAJEURE
The organisers are not liable for any claims for damages and/or losses if the entire conference has to be cancelled due to a force majeure incident.

DISCLAIMER
The organisers are not liable for damages and/or losses of any kind which may be incurred by the conference delegates or by any other individuals accompanying them, both during the official activities as well as going to/from the conference. Delegates are responsible for their own safety and belongings.

INSURANCE AND VACCINATIONS
The registration fee does not cover insurance for the delegates. The organisers recommend that delegates take out insurance in their home country to cover pre-journey cancellation for personal reasons and necessary insurance to cover accidents, medical expenses and loss of personal belongings during the visit. No vaccinations are needed when visiting Sweden.
## Sunday 3 July

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<td>15.00</td>
<td>Welcome</td>
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<td>Uppsala, Sweden</td>
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<td></td>
<td><strong>Chair persons</strong></td>
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<td>Robert Kisilevsky/Angela Dispenzier</td>
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<tr>
<td>15.15 – 16.00</td>
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<tr>
<td></td>
<td>Amyloid and amyloidosis</td>
<td>Giampaolo Merlini</td>
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<td>Pavia, Italy</td>
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<tr>
<td>16.00 – 18.00</td>
<td><strong>Basic science</strong></td>
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<td>16.00 – 16.30</td>
<td><strong>Keynote lecture</strong></td>
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<td>Human protein atlas</td>
<td>Fredrik Pontén</td>
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<td></td>
<td>for immunoglobulin light chain amyloidosis</td>
<td>Miguel Inacio Da Silva</td>
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<td>Filho Heidelberg,</td>
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<td></td>
<td></td>
<td>Germany</td>
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<td>16.45 – 17.00</td>
<td>The role of novel biomarkers in AL</td>
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<td>amyloidosis</td>
<td>Ga Yeon Lee</td>
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<td>Seoul, Korea</td>
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<td>17.00 – 17.15</td>
<td>Immunotherapy against alpha-synuclein</td>
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<td>protofibrils as a therapeutic strategy for</td>
<td>Joakim Bergström</td>
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<td>Parkinson’s disease</td>
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<td>17.15 – 17.30</td>
<td>siRNA targeting the D light chain constant</td>
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<td>region: A pre-clinical experimental approach to AL and non-fibrillar κ light chain deposition diseases</td>
<td>Xun Ma</td>
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<td>Boston, MA</td>
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<td>17.30 – 17.45</td>
<td>Structural stability and local dynamics in</td>
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<td>disease-causing mutants of human apolipoprotein A-I: what makes the protein amyloidogenic?</td>
<td>Olga Gursky</td>
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<td>17.45 – 18.15</td>
<td><strong>Special lecture</strong></td>
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<td>A backward glance at what gives us the</td>
<td>Robert A. Kyle</td>
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<td>future</td>
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<td></td>
<td>Rochester, MN</td>
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<tr>
<td>19.00</td>
<td><strong>Welcome reception</strong></td>
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<td><strong>Chair persons</strong></td>
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<td></td>
<td>Marcus Fändrich/Jeffrey W. Kelly</td>
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<tr>
<td>08.00</td>
<td><strong>Basic science</strong></td>
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<tr>
<td>08.00 – 08.30</td>
<td><strong>Keynote lecture</strong></td>
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<td></td>
<td>Chaperone in amyloidosis</td>
<td>Jan Johansson</td>
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<td>Stockholm, Sweden</td>
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<tr>
<td>08.30 – 09.00</td>
<td><strong>Keynote lecture</strong></td>
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<td></td>
<td>Amyloid and amylome</td>
<td>David Eisenberg</td>
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<td></td>
<td></td>
<td>Los Angeles, CA</td>
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<tr>
<td>09.00 – 09.15</td>
<td>A bifunctional peptide, “peptope”, for</td>
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<td>pre-targeting antibody 7D8 to systemic</td>
<td>Jonathan Wall</td>
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<td></td>
<td>amyloid deposits.</td>
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<td>Knoxville, TN</td>
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<tr>
<td>09.15 – 09.30</td>
<td>Acceleration of α-synuclein aggregation</td>
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<td>Ricardo Gaspar</td>
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<td>Lund, Sweden</td>
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<tr>
<td>Time</td>
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| 09.30 – 09.45| Carbamylation of the amino-terminal residue (Gly 1) of mouse SAA1.1 promotes amyloid fibril formation | O-13 | Barbara Kluve-Beckerman
|              | Indianapolis, IN                                                     |
| 09.45 – 10.15| Coffee break                                                         |
| 10.15 – 12.00| **Basic science**                                                    |
| 10.15 – 10.45| **Keynote lecture**                                                  |
|              | Seeding and cross-seeding                                            |
|              | O-14 | Christopher M. Dobson
|              | Cambridge, UK                                                        |
| 10.45 – 11.00| **Co-fibrillogenesis of wild-type and D76N β2-microglobulin seeding effect or prion-like mechanism?** | O-15 | Vittorio Bellotti
|              | Pavia, Italy and London, UK                                          |
| 11.00 – 11.15| **Molecular determinants of IAPP cross-amyloid interaction with Abeta** | O-16 | Aphrodite Kapurniotu
|              | Munich, Germany                                                      |
| 11.15 – 11.30| **Cross-seeding of medin and A-beta amyloid**                        | O-17 | Hannah Davies
|              | Liverpool, UK                                                        |
| 11.30 – 12.00| **Keynote lecture**                                                  |
|              | Treatment in pipeline                                                | O-18 | Sir Mark B. Pepys
|              | Liverpool, UK                                                        |
| 12.00 – 13.30| **Lunch and poster viewing**                                         |
| 13.30 – 15.00| **Imaging**                                                          |
| 13.30 – 14.00| **Keynote lecture**                                                  |
|              | *In vivo* detection of amyloid                                       | O-19 | Philip N. Hawkins
|              | London, UK                                                           |
| 14.00 – 14.15| **Regression of cardiac AL amyloidosis demonstrated by cardiovascular magnetic resonance: a new era of understanding** | O-20 | Ana Martinez Naharro
|              | London, UK                                                           |
| 14.15 – 14.30| **Visualisation of transthyretin heart amyloidosis by 11C-PiB and PET** | O-21 | Björn Pilebro
|              | Umeå, Sweden                                                         |
| 14.30 – 14.45| **Multicenter experience of planar technetium pyrophosphate cardiac imaging: Does preferential cardiac uptake predict survival in patients with ATTR cardiac amyloidosis?** | O-22 | Adam Castano
|              | New York, NY                                                         |
| 14.45 – 15.00| **Cardiac amyloid imaging with 18F-florbetaben positron emission tomography: a pilot study** | O-23 | Peter Mollee
|              | Queensland, Australia                                                |
| 15.00 – 15.30| **Coffee break**                                                    |
| 15.30 – 17.00| **Transthyretin**                                                    |
| 15.30 – 16.00| **Keynote lecture**                                                  |
|              | Transthyretin                                                         | O-24 | Merrill D. Benson
|              | Indianapolis, IN                                                    |
| 16.00 – 16.30| **Keynote lecture**                                                  |
|              | Antibodies in TTR-amyloidosis                                         | O-25 | Yukio Ando
|              | Kumamoto, Japan                                                      |
| 16.30 – 18.00| **Poster presentations**                                             |
| 19.00 – 21.00| **Satellite symposium**                                              |
|              | Alnylam                                                              | Main auditorium
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<td>08.00 – 09.30</td>
<td>Pathways for ATTR amyloid formation</td>
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<td>Presentations and discussion</td>
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<td>O-26 Vittorio Bellotti, Pavia, Italy and London, UK</td>
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<td>O-27 David Eisenberg, Los Angeles, CA</td>
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<td>O-28 Jeffrey W. Kelly, La Jolla, CA (Chair)</td>
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<td>O-29 Ole B. Suhr, Umeå, Sweden</td>
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<td>O-30 Per Westermark, Uppsala, Sweden</td>
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<td>Chair persons</td>
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<td>Yukio Ando/Marcia W. Cruz</td>
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<td>09.45 – 10.00</td>
<td>Establishment of a diagnostic center for amyloidosis in Japan by Kumamoto University</td>
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<td>O-31 Frederick Ruberg Boston, MA</td>
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<td>10.00 – 10.30</td>
<td>Coffee break</td>
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<td>10.30 – 10.45</td>
<td>Male gender is a risk factor for myocardial involvement in transthyretin-related amyloidosis: A study based on the Transthyretin Amyloid Outcomes Survey</td>
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<td>O-33 Claudio Rapezzi Bologna, Italy</td>
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<td>10.45 – 11.00</td>
<td>Unveiling transthyretin cardiac amyloidosis as an etiology for paradoxical low-flow severe aortic stenosis in patients undergoing transcatheter aortic valve replacement</td>
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<td>O-34 Adam Castano New York, NY</td>
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<td>11.00 – 13.00</td>
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<td>13.00 – 14.00</td>
<td>Lunch</td>
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<td>14.00 –</td>
<td>AL amyloidosis</td>
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<td>14.00 – 14.30</td>
<td>Keynote lecture</td>
<td>O-35 Raymond L. Comenzo Boston, MA</td>
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<td>AL amyloidosis</td>
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<td>14.30 – 14.45</td>
<td>Limited duration treatment with bortezomib and methylprednisone is effective in primary amyloidosis leading to results similar to those of high dose melphalan</td>
<td>O-36 Rafat Abonour Indianapolis, IN</td>
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<td>14.45 – 15.00</td>
<td>Renal outcome among patients with systemic AL amyloidosis who present with advanced CKD is dependent on speed and magnitude of response to chemotherapy</td>
<td>O-37 Tamer Rezk London, UK</td>
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<td>15.00 – 15.15</td>
<td>CS-1 chimeric antigen receptor adoptive T cell therapy for systemic light chain amyloidosis</td>
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<td>15.15 – 15.30</td>
<td>NEOD001 demonstrates organ biomarker responses in patients with light chain amyloidosis and persistent organ dysfunction: results from the expansion phase of a phase 1/2 study</td>
<td>O-39 Morie A. Gertz Rochester, MN</td>
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<td>17.00 – 18.00</td>
<td>Refreshments/dinner</td>
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<tr>
<td>18.00 – 19.30</td>
<td>Satellite symposium</td>
<td>Main auditorium</td>
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# Wednesday 6 July

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<tr>
<th>Time</th>
<th>Session</th>
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<tr>
<td>08.00 – 09.15</td>
<td>More AL amyloidosis</td>
<td>O-40 Morie A. Gertz, Rochester, MN</td>
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<td></td>
<td><strong>Debate</strong></td>
<td>O-41 Stephan Schönland, Heidelberg, Germany (Chair)</td>
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<td></td>
<td>Treatment of AL amyloidosis</td>
<td>O-42 Stephan Schönland, Heidelberg, Germany</td>
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<td></td>
<td>Stem cell transplantation or chemotherapy for front-line treatment of AL?</td>
<td>O-43 Martha Skinner, Boston, MA</td>
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<tr>
<td>09.15 – 09.30</td>
<td>A randomized phase iii trial of melphalan and dexamethasone (mdex) versus bortezomib, melphalan and dexamethasone (bmdex) for untreated patients with AL amyloidosis</td>
<td>See PB-86 Giovanni Palladini Pavia, Italy</td>
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<tr>
<td>09.30 – 10.00</td>
<td>Coffee break</td>
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<tr>
<td>10.00 – 11.00</td>
<td>AA amyloidosis</td>
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<td><strong>Chair persons</strong></td>
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<td></td>
<td>Steven Zeldenrust/Lawreen Connors</td>
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<tr>
<td>10.00 – 10.30</td>
<td><strong>Keynote lecture</strong></td>
<td>O-44 Bouke P.C. Hazenberg Groningen, the Netherlands</td>
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<tr>
<td></td>
<td>Pathogenesis and status of AA amyloidosis</td>
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<td>10.30 – 10.45</td>
<td>AA amyloidosis related to familial mediterranean fever: a cohort study of 31 cases</td>
<td>O-45 Sophie Georgin-Lavialle Paris, France</td>
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<td>10.45 – 11.00</td>
<td>Transcriptional upregulation and sequence variation in the SAA gene pathway in island foxes (<em>Urocyon littoralis</em>) with AA amyloidosis</td>
<td>O-47 Patricia Gaffney La Jolla, CA</td>
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<td>11.00 – 12.30</td>
<td><strong>Satellite symposium</strong></td>
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<td>Bellus</td>
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<tr>
<td>12.30 – 14.00</td>
<td>Lunch and Poster presentations</td>
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<td><strong>Chair persons</strong></td>
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<td></td>
<td>Arie Stangou/Keiichi Higuchi</td>
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<tr>
<td>14.00 – 15.05</td>
<td>More AA</td>
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<td></td>
<td><strong>Other amyloid forms</strong></td>
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<tr>
<td>14.00 – 14.15</td>
<td>A clinical Phase 3 confirmatory trial of Kiacta™ in the treatment of AA amyloidosis</td>
<td>O-48 Giampaolo Merlini Pavia, Italy</td>
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<tr>
<td>14.15 – 14.30</td>
<td>Passive treatment with monoclonal antibodies to islet amyloid polypeptide significantly improved both haemoglobin A1c and glucose tolerance and decreased extracellular amyloid deposits in the transgenic HIP rat model of type 2 diabetes</td>
<td>O-49 Wagner Zago San Francisco, CA</td>
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<tr>
<td>14.30 – 14.45</td>
<td>Prevalence of monoclonal gammopathy of unknown significance in ATTR-wild type patients</td>
<td>O-50 Hallie Geller Boston, MA</td>
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<td>14.45 – 15.05</td>
<td>Apolipoprotein C-II amyloidosis</td>
<td>O-51 Laura Obici, Pavia, Italy</td>
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<td>O-52 Samih Nasr, Rochester, MN</td>
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<td>15.05 – 15.30</td>
<td>Coffee break</td>
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<tr>
<td>15.30 – 17.30</td>
<td><strong>Parallel sessions:</strong></td>
<td>A. In Main auditorium</td>
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<td>A. Liver and heart transplantation in amyloidosis</td>
<td>B. In Hall B</td>
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<td>B. Animal models</td>
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<tr>
<td>15.30 – 17.30</td>
<td><strong>A. Liver and heart transplantation in amyloidosis</strong></td>
<td>Main auditorium</td>
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<td><strong>Chair persons</strong></td>
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<td>Bo-Göran Ericzon/Philip Hawkins</td>
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<td>15.30 – 16.00</td>
<td><strong>Keynote lecture</strong>&lt;br&gt;Liver transplantation</td>
<td>Bo-Göran Ericzon, Stockholm, Sweden</td>
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<tr>
<td>16.00 – 16.30</td>
<td><strong>Keynote lecture</strong>&lt;br&gt;Heart transplantation</td>
<td>Mathew Maurer, New York, NY</td>
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<td>16.30 – 16.45</td>
<td>Transthyretin-type cerebral amyloid angiopathy in post-transplant patients with hereditary ATTR amyloidosis: Correlates between clinical findings and PIB-PET imaging</td>
<td>Yoshiki Sekijima, Matsumoto, Japan</td>
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<td>16.45 – 17.00</td>
<td>Evaluation of central nervous complications following liver transplantation in patients with hereditary transthyretin amyloidosis</td>
<td>Nicklas Wange, Umeå, Sweden</td>
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<td>17.00 – 17.15</td>
<td>Polynephropathy after FAP domino liver transplantation</td>
<td>Göran Solders, Stockholm, Sweden</td>
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<td>17.15 – 17.30</td>
<td>Cardiac dysautonomia predicts long term survival in hereditary transthyretin amyloidosis after liver transplantation</td>
<td>Michel Slama, Paris, France</td>
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<tr>
<td>16.30 – 17.30</td>
<td><strong>B. Animal models</strong>&lt;br&gt;Presentations and discussion: Experimental animal models</td>
<td>Joel N. Buxbaum, La Jolla CA (Chair)</td>
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<td>Chair persons</td>
<td>Jean D. Sipe/Joel Buxbaum</td>
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<td>Jean D. Sipe/Joel Buxbaum</td>
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<tr>
<td>15.30 – 16.45</td>
<td>Exploring the mechanism of cardiotoxicity in AL amyloidosis: the C. elegans model</td>
<td>Paola Rogroni, Pavia, Italy</td>
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<td>16.45 – 17.00</td>
<td>Efficiency of siRNA for removal of transthyretin V30M in a TTR leptomeningeal animal model</td>
<td>Maria J. Saraiva, Porto, Portugal</td>
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<tr>
<td>17.00 – 17.15</td>
<td>Serum amyloid A in the treatment of sepsis in a mouse model</td>
<td>Reinhold P. Linke, Martinsreid, Germany</td>
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<td>19.00</td>
<td><strong>Dinner at Uppsala Castle</strong></td>
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### Thursday 7 July

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<td>08.00 – 10.00</td>
<td><strong>Parallel sessions</strong></td>
<td><strong>General discussions</strong></td>
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<tr>
<td>08.00 – 09.15</td>
<td><strong>Discussion I</strong></td>
<td>Suitable methodology for treatment evaluation</td>
<td>O-66 Teresa Coelho; Porto, Portugal&lt;br&gt; O-67 Thibaud Damy, Paris, France&lt;br&gt; O-68 Steven Zeldenrust, Rochester, MN (Chair)</td>
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<tr>
<td>09.15 – 09.30</td>
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<td>Chair persons: Teresa Coelho/Raymond Comenzo</td>
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<td>09.30 – 09.45</td>
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<td>The 6-minute walk test in AL amyloidosis patients: a single center experience</td>
<td>O-69 Vina Pulido Boston, MA</td>
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<tr>
<td>09.45 – 10.00</td>
<td></td>
<td>Lessons for future clinical trial design in cardiac amyloidosis - the experience of a prospective study in stage III cardiac AL amyloidosis (REVEAL study)</td>
<td>O-70 Ashutosh Wechalekar, London, UK</td>
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<tr>
<td>10.00 – 10.15</td>
<td></td>
<td>Evaluation of Therapeutic Oligonucleotides for Familial Amyloid Polyneuropathy in a Stem Cell-Based in vitro Model</td>
<td>O-71 Christoph Niemietz Munster, Germany</td>
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<tr>
<td>08.00 – 09.15</td>
<td><strong>Discussion II</strong></td>
<td>ThT assay pitfalls- can the method be standardised?</td>
<td>O-73 Jeffrey W. Kelly, La Jolla, CA&lt;br&gt; O-74 Hironobu Naiki, Fukui, Japan&lt;br&gt; O-75 Sofie Nyström, Linköping, Sweden&lt;br&gt; O-76 Daniel Otzen, Aarhus, Denmark (Chair)</td>
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<td>09.15 – 09.30</td>
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<td>Chair persons: Daniel Otzen/Per Westermark</td>
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<td>09.30 – 09.45</td>
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<td>FRET-based observation of intracellular SAA fibril formation</td>
<td>O-77 Stephanie Claus Ulm, Germany</td>
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<td>09.45 – 10.00</td>
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<td>First report of MYD88 L265P somatic mutation in IgM-associated light chain amyloidosis</td>
<td>O-78 Rajshekhar Chakraborty Rochester, MN</td>
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<td>10.00 – 10.15</td>
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<td>Cell damage in light chain amyloidosis: fibril internalization, toxicity and cell-mediated seeding</td>
<td>O-79 Marta Marin-Argany Knoxville, TN</td>
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<td>Discovery of transthyretin stabilizers displaying resilience to the effect of the V30M mutation</td>
<td>O-80 Rui Brito Porto, Portugal</td>
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<tr>
<td>10.15 – 10.45</td>
<td><strong>Coffee</strong></td>
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<tr>
<td>11.30 – 12.00</td>
<td><strong>Summary of the meeting</strong></td>
<td><strong>Main auditorium</strong></td>
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<td>Martha Skinner Boston, MA</td>
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<td>12.00</td>
<td><strong>Closing remarks</strong></td>
<td>Gunilla T. Westermark Ole B. Suhr Per Westermark</td>
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<tr>
<td>12.30 – 13.30</td>
<td><strong>Grab &amp; go lunch</strong></td>
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ISA 2016

1. Uppsala Central station
2. Tourist information
3. Uppsala Konsernt & Kongress, UKK
   Address: Vaksala torg 1
4. Uppsala Botanical Garden
   Address: Villavägen 8
5. Best Western Hotel Svava
   Address: Bangårdsgatan 24
6. Radisson Blu Hotel Uppsala
   Address: Stationsgatan 4
7. Park Inn by Radisson
   Address: Storgatan 30
8. Uppsala castle
   Address: Entrance H0

Welcome reception July 3
Conference Dinner July 6
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| O3 | Identifying Germline Genetic Risk Factors For Immunoglobulin Light Chain Amyloidosis |
| O4 | The Role Of Novel Biomarkers In AL Amyloidosis |
| O5 | Immunotherapy Against Alpha-Synuclein Prototibrils As A Therapeutic Strategy For Parkinson’s Disease |
| O6 | SiRNA Targeting The K Light Chain Constant Region: A Pre-Clinical Experimental Approach To AL And Non-Fibrillar K Light Chain Deposition Diseases |
| O7 | Structural Stability And Local Dynamics In Disease-Causing Mutants Of Human Apolipoprotein A-I: What Makes The Protein Amyloidogenic? |
| O9 | The BRICHOS Domain: An Anti-Amyloid Chaperon |
| O16 | Molecular Determinants Of IAPP Cross-Amyloid Interaction With Abeta |
| O17 | Cross-Seeding Of Medin And A-Beta Amyloid |
| O18 | Immunotherapeutic Clearance Of Systemic Amyloid Deposits By Antibodies To Serum Amyloid P Component |
| O20 | Regression Of Cardiac AL Amyloidosis Demonstrated By Cardiovascular Magnetic Resonance: A New Era Of Understanding |
| O21 | Visualisation Of Transthyretin Heart Amyloidosis By 11C-PIB And PET |
| O22 | Multicenter Experience Of Planar Technetium Pyrophosphate Cardiac Imaging: Does Preferential Cardiac Uptake Predict Survival In Patients With ATTR Cardiac Amyloidosis? |
| O23 | Cardiac Amyloid Imaging With 18F-Florbetaben Positron Emission Tomography: A Pilot Study |
| O24 | Transthyretin Amyloidosis |
| O25 | Antibody Therapy For Transthyretin Related Familial Amyloid Polyneuropathy-Another Therapeutic Option |
| O28 | Preparation Of Misfolded TTR Oligomers Under Physiological Conditions In Vitro And A Preliminary Structure–Proteotoxicity Relationship Study |
| O29 | Clinical Implications Of Amyloid Fibril Composition In ATTR-Amyloidosis |
| O30 | Domino Liver Transplantation: Full-Length Transthyretin In Donor And Recipient Patients With ATTR Val30Met Amyloidosis |
| O31 | Retinol Binding Protein 4 (RBP4) Concentration Identifies V122I Transthyretin Cardiac Amyloidosis |
| O32 | Establishment Of A Diagnostic Center For Amyloidosis In Japan By Kumamoto University |
| O33 | Male Gender Is A Risk Factor For Myocardial Involvement In Transthyretin-Related Amyloidosis: A Study Based On The Transthyretin Amyloid Outcomes Survey |
| O34 | Unveiling Transthyretin Cardiac Amyloidosis As An Etiology For Paradoxical Low-Flow Severe Aortic Stenosis In Patients Undergoing Transcatheter Aortic Valve Replacement |
| O36 | Limited Duration Treatment With Bortezomib And Methylprednisone Is Effective In Primary Amyloidosis Leading To Results Similar To Those Of High Dose Melphalan |
| O37 | Renal Outcome Among Patients With Systemic AL Amyloidosis Who Present With Advanced CKD Is Dependent On Speed And Magnitude Of Response To Chemotherapy. |
| O38 | Cs-1 Chimeric Antigen Receptor Adoptive T Cell Therapy For Systemic Light Chain Amyloidosis |
| O39 | NEOD001 Demonstrates Organ Biomarker Responses In Patients With Light Chain Amyloidosis And Persistent Organ Dysfunction: Results From The Expansion Phase Of A Phase 1/2 Study |
| O41 | AL Amyloidosis Can Be Treated Effectively With Non-Transplant Therapy |
| O43 | High-Dose Melphalan And Autologous Stem Cell Transplantation (Hdm/Sct) As Frontline Treatment For Al Amyloidosis |
| O45 | AA Amyloidosis Related To Familial Mediterranean Fever: A Cohort Study Of 31 Cases. |
| O46 | A randomized phase iii trial of melphalan and dexamethasone (mdex) versus bortezomib, melphalan and dexamethasone (bmdex) for untreated patients with AL amyloidosis |
| O47 | Transcriptional Upregulation And Sequence Variation In The SAA Gene Pathway In Island Foxes (Urocyon Littoralis) With AA Amyloidosis |
| O48 | A Clinical Phase 3 Confirmatory Trial Of Kiactatm In The Treatment Of AA Amyloidosis |
| O49 | Passive Treatment With Monoclonal Antibodies To Islet Amyloid Polypeptide Significantly Improved Both Hemoglobin A1c And Glucose Tolerance And Decreased Extracellular Amyloid Deposits In The Transgenic Hip Rat Model Of Type 2 Diabetes |
| O50 | Prevalence Of Monoclonal Gammopathy Of Unknown Significance In Attr-Wild Type Patients |
| O51 | Amyloidosis Derived From Apolipoprotein C-II: A Novel Type Of Renal Amyloidosis |
| O52 | Renal Amyloidosis Associated With A Novel ApoC-II Variant |
| O53 | Liver Transplantation In Treatment Of TTR Amyloidosis |
| O54 | Cardiac Transplantation for Amyloidosis |
| O55 | Transthyretin-Type Cerebral Amyloid Angiopathy In Post-Transplant Patients With Hereditary ATTR Amyloidosis: Correlates Between Clinical Findings And PIB-PET Imaging |
| O56 | Evaluation Of Central Nervous Complications Following Liver Transplantation In Patients With Hereditary Transthyretin Amyloidosis |
| O57 | Polyneuropathy After FAP Domino Liver Transplantation |
| O58 | Cardiac Dysautonomia Predicts Long Term Survival In Hereditary Transthyretin Amyloidosis After Liver Transplantation |
| O59 | Animal Models Of The Human Amyloidoses Revisited |
| O60 | Reproducing Light Chain Proteotoxicity In Animal Models: A Window On AL Cardiomyopathy |
| O61 | Animal Models Of Transthyretin Amyloidosis To Search For FAP Patient’S Biomarkers |
| O62 | Drosophila Melanogaster As A Model In Protein Aggregation Diseases |
| O63 | Exploring The Mechanism Of Cardiotoxicity In Al Amyloidosis: The C. Elegans Model |
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| O65 | Serum Amyloid A In The Treatment Of Sepsis In A Mouse Model. |
| O69 | The 6-Minute Walk Test In AL Amyloidosis Patients: A Single Center Experience |
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| O71 | Evaluation Of Therapeutic Oligonucleotides For Familial Amyloid Polyneuropathy In A Stem Cell-Based In Vitro Model |
| O72 | Treatment Of Transthyretin Cardiomyopathy With A TTR Specific Antisense Oligonucleotide (IONIS-TTR Rx) |
| O73 | Detection Of Circulating Misfolded TTR Oligomers In Ttr Polyneuropathy And Cardiomyopathy Patients And A Reduction In Levels Upon Tafamidis Treatment |
| O74 | Thioflavin T: Not An All-Rounder, But A Trustworthy Friend For Over 27 Years |
| O75 | Seed-Dependent Templating Of Murine AA Amyloidosis |
| O77 | Fret-Based Observation Of Intracellular SAA Fibril Formation |
| O78 | First Report Of MYD88 L265P Somatic Mutation In IgM-Associated Light Chain Amyloidosis |
| O79 | Cell Damage In Light Chain Amyloidosis: Fibril Internalization, Toxicity And Cell-Mediated Seeding |
| O80 | Discovery Of Transthyretin Stabilizers Displaying Resilience To The Effect Of The V30M Mutation |
AMYLOID ANDamyloidosis

Giampaolo Merlini

Amyloidosis Research and Treatment Center, Foundation IRCCS Policlinico San Matteo, University of Pavia, Italy

ABSTRACT

In systemic amyloidoses, fibril precursors are produced most commonly by the liver or bone marrow; are released to plasma, and, by partly-known mechanisms, are predominantly trapped at certain sites, where they assemble into amyloid fibrils causing organ dysfunction and damage, eventually leading to death [1]. The key issue in amyloid research for developing targeted therapies is the definition of the mechanisms of organ targeting and damage. After disease initiation, the complexity of the downstream pathogenic processes increases and mechanisms of organ damage may be different for the various amyloid diseases.

Amyloid fibrils are the pathological hallmark in patients suffering from amyloidosis; thus, the fibrils themselves have long been considered the main pathogenic agent. The presence of amyloid deposits physically distorts the macroscopic and microscopic tissue architecture and thereby compromise organ functions via simple mass action [2]. Whilst mass action may be predominant in some systemic amyloidoses, such as transthyretin and apolipoprotein-AI amyloidosis, and for cerebral amyloid angiopathy, many research groups have found in vitro, in vivo, and clinical evidence that entities of smaller molecular weight, termed amyloid oligomers or prefibrillar oligomers, could be the main pathogenic agents in brain amyloid diseases [3] and in certain systemic amyloidoses [1]. Amyloid oligomers are less ordered than the fibrils and may expose hydrophobic regions and unpaired β-strands that confer a high potential to interact with other cellular factors, including proteins, membrane structures (both receptor-mediated and nonreceptor-mediated membrane interactions) and organelles, particularly mitochondria. Thus, they cause toxicity by a dominant mechanism. The capacity of the proteostasis network to deal with dangerous protein species declines during ageing, providing a potential target for pharmacological intervention. Amyloid deposits are not benign as amyloid fibres can disrupt membranes, catalyze the formation of cytotoxic oligomers and serve as a source of the more toxic and soluble assemblies, consistent with the view that a dynamic continuum of the various amyloid intermediates elicits toxicity. In addition, the advent of exome and genome sequencing have revealed other possible players in the pathogenic processes, such as cholesterol metabolism, the innate immune system, and endosomal vesicle recycling [3]. This increasing complexity provides further targets for therapeutic interventions. It is not a question of one hypothesis against another, rather, we must pursue multiple approaches that may together prevent the end organ damage.

Immunoglobulin light chain (AL) amyloidosis, the most common form of systemic amyloidosis, exemplifies these principles. The possibility to accurately measure the circulating amyloid precursor (free light chains) and sensitive biomarkers of end organ damage, has illuminated the pathogenesis of this rapidly progressing disease. The prompt elimination of the toxic amyloid precursor, thanks to ever more effective chemo- and immune-therapies, results in parallel reduction of the biomarkers, rapid clinical improvement and markedly prolonged survival, despite the substantial persistence of amyloid deposits. Animal models have allowed the discovery of novel agents attenuationg the light chain toxicity. Immunotherapies targeting the amyloid deposits are entering the clinic. The combination of these multiple approaches is expected to accelerate the recovery of end organ damage and change the face of AL amyloidosis [4].

REFERENCES:

Identifying germline genetic risk factors for immunoglobulin light chain amyloidosis

MI da Silva Filho1, I Meziane1, C Campo1, N Weinhold2,3, S Huhn2, G Palladini4, Ashu Wechalekar5, U Hegenbart2, G Merlini4, A Försti1,6, S Schönland2, K Hemminki1,6

1 Division of Molecular Genetic Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany. 2 Department of Internal Medicine V, University of Heidelberg, Heidelberg, Germany. 3 Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, Little Rock, AR, USA. 4 Amyloidosis Research and Treatment Center, Biotechnology Research Laboratories, Department of Molecular Medicine, Fondazione IRCCS Policlinico San Matteo and University of Pavia, Pavia, Italy. 5 National Amyloidosis Centre, Division of Medicine, University College London, London, UK. 6 Center for Primary Health Care Research, Lund University, Malmo, Sweden.

m.dasilvafilho@dkfz-Heidelberg.de

INTRODUCTION: Immunoglobulin light-chain (AL) amyloidosis is a monoclonal plasma cell disease characterized by tissue deposition of misfolded immunoglobulin light-chains leading to progressive dysfunction of various organs, including heart, kidney and liver. Around 10 to 15% of multiple myeloma (MM) patients have AL. Three genome-wide association studies (GWASs) identified 8 different genetic risk loci associated with predisposition to MM [1-3]. Although 7 of those single nucleotide polymorphisms (SNPs) also impact AL amyloidosis [4], no genetic risk loci specific to this disease have been identified to date. To identify such loci we conducted a GWAS on AL amyloidosis patients. Genetic relationship between AL amyloidosis and MM was also further investigated by comparing the present AL results to the GWAS data of MM.

MATERIAL & METHODS: A GWAS was conducted using DNA samples from a total of 1351 AL amyloidosis patients from three independent cohorts of German, Italian and UK origin. For the comparison with MM we included data for 1508 German and 2282 UK MM patients. Analyses were conducted using PLINK v1.07 and R 2.15. Data underwent a strict quality control protocol. Unphenotyped SNPs were imputed using the 1000 Genomes Project (phase 3, Oct. 2014) as reference. Association tests were performed with SNPTESTv2.5 under a probabilistic dosage model to account for imputation uncertainty. Meta-analysis was conducted under a fixed model using the software GWAMA. Functional consequences of SNPs were inquired using available bioinformatics tools such as HaploReg and Regulome.

RESULTS: We identified 10 regions associated with AL amyloidosis. Five of these loci also significantly impacted susceptibility to MM. The other 5 SNPs showed detectable associations in AL amyloidosis only, with one of them reaching genome-wide significance.

DISCUSSION & CONCLUSIONS: Results of the present work give further support for the shared inherited predisposition between AL amyloidosis and MM. It also shows that AL amyloidosis specific germline risk loci do exist. Further work is necessary to confirm these results and to clarify their functional consequences.

The role of novel biomarkers in AL amyloidosis

Ga Yeon Lee1, Jin-Oh Choi1, Kihyun Kim2, Seok Jin Kim2, Hee-Jin Kim3, Soo-Youn Lee3, Eun-Seok Jeon1

1Division of Cardiology, Department of Medicine, 2Division of Hematooncology, Department of Medicine, 3Department of Laboratory Medicine & Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

gyneo.lee@gmail.com

INTRODUCTION: Cardiovascular biomarkers including troponin T (TnT) and N-terminal pro natriuretic peptide (NT-pro BNP) are well known as powerful prognostic factors in light chain (AL) amyloidosis patients. However, there is a lack of data regarding the role of novel biomarkers in AL amyloidosis patients. We investigated the role of soluble ST2 (sST2), growth differentiation factor 15 (GDF15), and osteopontin in AL amyloidosis.

MATERIALS & METHODS: From 2010 to 2015, total 73 AL amyloidosis patients agreed to provide their serum at the diagnosis of AL amyloidosis for the research. The level of sST2, GDF15, and osteopontin were measured simultaneously and analyzed with other clinical characteristics. The median follow-up duration was 18.0 (12.4-28.1) months.

RESULTS: Total 25 deaths occurred during the follow-up period. The cut-off values derived from ROC curve to predict one year mortality were 32.6 ng/mL (area under the curve, AUC 0.72 with sensitivity 90.5% and specificity 51.9%) in sST2, 2.30ng/mL (AUC 0.72 with sensitivity 71.4% and specificity 44.2%) in GDF15, and 154.7ng/mL (AUC 0.64 with sensitivity 52.4% and specificity 75.0%) in osteopontin. In Cox proportional hazard regression analysis, the patients with elevated levels of those biomarkers (sST2 ≥32.6ng/mL, GDF15 ≥2.30ng/mL, or osteopontin≥154.7ng/mL) showed significantly poor prognosis in all-cause mortality [hazard ratio, HR 6.16 (1.84-20.64), p=0.003, 3.82 (1.68-8.72), p=0.001, 2.26 (1.02-5.01), p=0.044, respectively].

DISCUSSIONS & CONCLUSIONS: Novel cardiovascular biomarkers seem to have additional prognostic value in AL amyloidosis patients. Further larger studies will be required to confirm these findings.

Fig.1: Survival curves according to sST2, GDF-15 and Osteopontin
Immunotherapy against alpha-synuclein protofibrils as a therapeutic strategy for Parkinson’s disease

J Bergström¹, G Gustafsson¹, V Lindström¹, T Outeiro², F Eriksson³, E Nordström³, J Sigvardsson³, M Johannesson³, S Tucker³, C Möller³, L Lannfelt¹, M Ingelsson¹

¹Department of Public Health and Caring Sciences, Uppsala University, Uppsala, Sweden.
²Department of Neurodegeneration and Restorative Research, University Medical Center Göttingen, Göttingen, Germany. ³BioArctic Neuroscience, Stockholm, Sweden.

INTRODUCTION: Alpha-synuclein is the main component of intraneuronal fibrillar inclusions, Lewy bodies, found in the Parkinson’s disease brain. In recent years, animal studies have indicated immunotherapy with antibodies directed against alpha-synuclein as a promising novel treatment strategy for Parkinson’s disease. Since alpha-synuclein protofibrils have been demonstrated to possess pronounced cytotoxic properties, such species should be particularly attractive as therapeutic targets. We have generated alpha-synuclein protofibril-selective monoclonal antibodies and studied by which mechanisms such antibodies can be internalized by living cells. Furthermore, it was investigated if immunotherapy with such antibodies can reduce brain pathology in an alpha-synuclein transgenic mice model.

MATERIAL & METHODS: Human neuroglioma H4 cells were treated with either the monoclonal protofibril selective alpha-synuclein antibody mAb47 or control antibodies. The uptake was evaluated by fluorescence microscopy. For the in vivo studies, (Thy-1)-h[A30P] alpha-synuclein transgenic mice were used. Such mice display alpha-synuclein pathology in CNS as well as behavioral and motor symptoms at 15-18 months of age. Fourteen-month-old mice were treated for 14 weeks with weekly intraperitoneal injections of mAb47. Next, the brain tissue was evaluated by biochemical and histochemical techniques.

RESULTS: In our cell system, mAb47 displayed a robust cellular uptake, already after four hours of antibody exposure. An increased internalization was observed in alpha-synuclein overexpressing cells compared to non-transfected cells. Similarly, the presence of alpha-synuclein in the media facilitated a greater uptake of the mAb47 antibody. Finally, different Fcγ receptors were targeted and blockage of FcγRI and FcγRIIB/C were shown to reduce antibody uptake. For the in vivo part, the transgenic alpha-synuclein mice displayed lower levels of both soluble and membrane-associated alpha-synuclein protofibrils in the spinal cord, as compared to placebo treated mice. In contrast, no effect on monomeric or fibrillar levels of alpha-synuclein was observed. Furthermore, no increase in inflammation was seen following antibody treatment.

DISCUSSION AND CONCLUSIONS: Taken together, immunotherapy with antibodies targeting soluble aggregated, toxic alpha-synuclein species holds promise as a future disease-modifying treatment in Parkinson’s disease.
siRNA targeting the κ light chain constant region: A pre-clinical experimental approach to AL and non-fibrillar κ light chain deposition diseases

Xun Ma1, Ping Zhou1, Sandy W Wong1, Melissa Warner1, Chakra Chaulagain2, Raymond L Comenzo1

1The John C Davis Myeloma and Amyloid Program, Tufts Medical Center, Boston, MA, USA. 2Taussig Cancer Institute of Cleveland Clinic, Cleveland Clinic Florida, Weston, FL, USA

xma@tuftsmedicalcenter.org

Treatment of light chain (LC) deposition diseases both fibrillar and non-fibrillar is aimed at eliminating LC production but success is limited. The LC causing systemic light-chain amyloidosis (AL) can lead to early cardiac-related deaths in up to one quarter of patients, and those associated with myeloma (MM) causing non-fibrillar LC deposition disease frequently result in dialysis-dependent renal failure. Despite recent advances, incurability, progressive organ damage, and in AL early cardiac deaths, continue to dominate the clinical scenarios of these LC deposition diseases, making the pursuit of more effective therapies a priority.

We report our experimental results by using a designed siRNA pool targeting the κ LC constant region mRNA against all κ plasma cell clones (si[IGKC]). To design si[IGKC] we mined the IMGT database for all expressed κ LC gene sequences, examined their constant regions and identified consensus sequences exclusive of polymorphic nucleotides in Km allotype codons. We introduced the siRNA pool into the kappa LC producing plasma cells both in vitro and in vivo and measured changes of κ LC mRNA and protein production by means of RT-qPCR, immunoblot, and ELISA assay for κ LC secretion.

In vitro and in vivo there were statistically significant reductions in κ LC message and protein production by all modalities in all cell types despite diversity in variable region sequence. In vitro we employed 4 human MM cell lines that make κ LC; reductions in κ LC message and protein production after 1 day are shown in Figures A and B with quadruplicated repeats for each cell line. In 20 specimens of CD138-selected marrow plasma cells from patients with κ plasma cell diseases (AL=11, MM=9), averaged reductions in κ LC mRNA and in κ LC secretion after 1 day were 60% and 30% respectively. The average amount of κ LC per million cells/ml in 1 day in culture was 10.2μg, compared to the cell lines that secreted an average of 3.5μg; the reduction in secretion by patient specimens treated with si[IGKC] averaged 3μg. In vivo we employed flank plasmacytoma xenograft model (NCI-H929 cells) in NSG mice. IB of 7 si[-]control and 7 si[IGKC] treated mice with relative κ LC densities are shown in Figure C. The significant correlation (r=0.80, P<0.01) of κ LC densities with fold changes in mRNA is shown in Figure D. Human κ LC levels in serum of NCI-H929 xenografted mice at baseline(pre-) and 4 days later at sacrifice(post-) for both groups were significantly different. Controls had 6.09 +/-2.48mg/mL pre- and 7.27 +/- 2.26μg/mL post- (mean +/- SD, P=0.42, paired t test) and si[IGKC] treated mice had 8.99 +/- 8.08 μg/mL pre- and 4.58 +/- 5.11μg/mL post- (P=0.02), a 50% reduction in circulating κ LC.

In conclusion, si[IGKC] can significantly reduce κ LC production by κ plasma cells. Further pre-clinical development is needed to optimize delivery.
Structural stability and local dynamics in disease-causing mutants of human apolipoprotein A-I: what makes the protein amyloidogenic?

M Das,1 CJ Wilson,2 X Mei,1 T Wales,2 JR Engen,2 O Gursky1

1 Department of Physiology & Biophysics, Boston University School of Medicine, Boston USA.
2 Department of Chemistry, Northeastern University, Boston, USA.

Gursky@bu.edu

INTRODUCTION: Apolipoprotein A-I, the major protein of plasma high-density lipoprotein (HDL, or “good cholesterol”), removes cellular cholesterol and protects against atherosclerosis. ApoA-I mutations can cause either aberrant lipid homeostasis or familial amyloidosis, a life-threatening disease wherein N-terminal protein fragments form fibrils in vital organs.

MATERIALS & METHODS: To unveil the protein misfolding mechanism and to understand why some protein destabilizing mutations cause amyloidosis while others do not, we analyzed the structure, stability and lipid-binding properties of naturally occurring mutants of full-length human apoA-I causing either amyloidosis (G26R, W50R, F71Y, L170P) or aberrant lipid metabolism (L159R). Global and local protein conformation and dynamics in solution were assessed by far- and near-UV circular dichroism spectroscopy, Tyr and ANS fluorescence, and hydrogen-deuterium exchange mass spectrometry.

RESULTS: All mutants showed increased deuteration in residues 14-22, supporting our hypothesis that decreased protection of this major amyloid “hot spot” can trigger protein misfolding. In addition, L159R showed local helical unfolding near the mutation site, consistent with cleavage of this mutant in plasma to generate the labile 1-159 fragment. Together, the results suggest that reduced protection of the major amyloid “hot spot”, combined with structural integrity of the native helix-bundle conformation, shift the balance from protein clearance to β-aggregation.

DISCUSSION & CONCLUSIONS: A delicate balance between the overall structural integrity of a globular protein and the local destabilization of its amyloidogenic segments may be a fundamental determinant of this and other amyloid diseases. Furthermore, mutation-induced conformational changes observed in the helix bundle, which comprises N-terminal 75% of apoA-I, and its flexible C-terminal tail suggest the propagation of structural perturbations to distant sites via an unexpected template-induced ensemble-based mechanism, challenging the classical structure-based view.

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Fig. 1 Binding to HDL stabilizes the apoA-I structure against misfolding or proteolysis, while the dissociated apoA-I is labile. If a mutation promotes apoA-I cleavage and formation of unstable fragments, the protein is rapidly cleared from circulation (top right). If a mutation increases exposure of amyloidogenic segments without producing unstable fragments, the protein forms amyloid (bottom).
The BRICHOS domain: an anti-amyloid chaperone

Jan Johansson

Department of Neurobiology, Care Sciences and Society, Center for Alzheimer Research, Karolinska Institutet, Novum, 141 57 Huddinge, Sweden

ABSTRACT
The BRICHOS domain has a unique structure and is part of several human type II transmembrane (TM) proproteins associated with proliferative, degenerative and amyloid diseases. All BRICHOS containing proproteins contain regions that are prone to form β-sheet aggregates, and prosurfactant protein C (proSP-C) BRICHOS has been shown to work as an intra-molecular chaperone for the highly aggregation-prone TM mature SP-C part. This BRICHOS function apparently prevents SP-C from forming amyloid, since mutations in proSP-C BRICHOS give rise to lung fibrosis with amyloid plaques composed of mature SP-C. Mutations in the BRICHOS containing Bri2 protein is associated with familial British and Danish dementias, in which the peptides ABri and ADan form amyloid. Both Bri2 and proSP-C BRICHOS domains have been shown not only to prevent their respective physiological client peptides from forming amyloid, but also to markedly reduce fibril formation and toxicity of the Alzheimer disease (AD) associated amyloid β-peptide (Aβ). Bri2 BRICHOS co-localizes with senile plaques in AD brains. The proSP-C BRICHOS domain specifically blocks the secondary nucleation process of Aβ42 fibril formation, while Bri2 BRICHOS blocks both the secondary nucleation and elongation steps. These observations suggest that the BRICHOS domain is the first described example of endogenous molecular chaperones that have evolved to specifically inhibit formation of amyloid, and in particular prevent the cell toxicity associated with amyloid fibril formation. BRICHOS can prevent toxicity of Aβ42 not only in vitro but also in animal models of AD, and it therefore holds potential as a novel treatment strategy against this most devastating amyloid disease.

The small heat chock protein (sHSP) αB crystalline, like Bri2 BRICHOS, blocks both the secondary nucleation and elongation steps of Aβ42 fibril formation, while the molecular chaperone DnaJB6 specifically blocks primary nucleation. This raises questions whether BRICHOS, like HSPs, also generally prevent partly unfolded proteins from forming amorphous aggregates. Recombinant human Bri2 BRICHOS domain but not recombinant human proSP-C BRICHOS is an efficient general molecular chaperone that prevents aggregation of the thermally denatured model substrates citrate synthase, luciferase and rhodaese. Moreover, Bri2 BRICHOS, like sHSPs, form oligo- and multimeric structures and the size of the oligomers apparently correlate with the efficiency as a molecular chaperone.

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Inhibiting Amyloidosis
David Eisenberg, Lorena Saelices, Michal R. Sawaya, Alice Soragni
Sanaz Memarzadeh, Duilio Cascio, Lisa Johnson
HHMI-UCLA david@mbi.ucla.edu

ABSTRACT
On the assumption that inhibiting amyloid fibril formation can halt the progression of amyloid diseases, we have taken a structure-based approach to the design of inhibitors. For a given disease-related, amyloid-forming protein, we first identify the adhesive segment(s) that cause the protein to enter the amyloid state. The identification is made by the 3D Profile computer algorithm (Bowie et al. 1991; Goldschmidt et al. 2000), followed by in vitro validation that the segment indeed forms amyloid fibrils. Then the 3D atomic structure of the amyloid fibrils formed by the segment is determined by X-ray or electron crystallography (Sawaya et al. 2007; Rodriguez et al. 2015). Next the known atomic structure is used as a template for design of a peptide inhibitor (Sievers et al. 2011), and the inhibitor is tested in vitro for inhibition of the fibrils of the segment and next for inhibition of fibrils of the parent protein, containing the segment. For intracellular amyloid-forming proteins, the inhibitor is rendered cell penetrating. If possible, the inhibitor is tested in an animal model of the corresponding amyloid disease. To date, this procedure has been most fully developed to halt p53 aggregation in ovarian cancer (Soragni et al. 2016) and transthyretin aggregation in transthyretin amyloidosis (Saelices et al. 2015).

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A BIFUNCTIONAL PEPTIDE, “PEPTOPE”, FOR PRE-TARGETING ANTIBODY 7D8 TO SYSTEMIC AMYLOID DEPOSITS.

JS Wall1,2, A Williams1, JS Foster1, EB Martin1, T Richey1, A Stuckey1, S Macy1, W Zago3, GG Kinney3 and SJ Kennel1,2

1 Department of Medicine, University of Tennessee Medical Center, Knoxville, USA. 2 Department of Radiology, University of Tennessee Medical Center, Knoxville, USA. 3 Prothena Biosciences, San Francisco, USA

jwall@utmck.edu

INTRODUCTION: At present there are two amyloid fibril-reactive antibodies in clinical trial for the treatment of patients with systemic amyloidosis light chain-associated (AL) amyloidosis. In an effort to potentially enhance the utility of these antibodies, we have developed a system that would allow these reagents to be used as immunotherapies for patients with other kinds of systemic amyloidosis, notably patients with transthyretin-associated (ATTR) amyloidosis. We have synthesized a bi-functional peptide (peptope) that comprises the pan-amyloid reactive peptide, p5, with a linear epitope recognized by the 7D8 monoclonal antibody (mAb), which specifically targets AA and light chain amyloid. The peptope was designed to facilitate the binding of mAb 7D8 with amyloid deposits for which it did not display a natural reactivity, e.g., ATTR. The use of a bifunctional peptope may enhance and expand the utility of mAbs already present in the clinical space for the treatment and removal of diverse amyloid deposits, regardless of the precursor protein from which they are comprised.

MATERIALS & METHODS: A panel of peptopes was synthesized using peptide p5 as the amyloid-reactive sequence, joined via one of four linker sequences to the –HEDT-COO- epitope sequence recognized by mAb 7D8. The binding of each peptope to amyloid and mAb 7D8 was measured separately by ELISA and surface plasmon resonance, to ensure that both functions were preserved in the context of the peptope. Peptope-mediated binding of 125I-labeled 7D8 mAb to synthetic fibrils composed of Aβ(1-40) or human IAPP, as well as human ATTR amyloid extracts (non-natural targets of the mAb) was performed in the presence an absence of peptope using a solution phase “pulldown” assay and by ELISA. Reactivity with ATTR amyloid in formalin-fixed paraffin embedded (FFPE) tissue sections was assayed suing biotinyl-7D8 with or without peptope pretreatment. Ex vivo phagocytosis assays were performed using pHrodo green-conjugated amyloid extracts and Raw 246.7 cells.

RESULTS: The 7D8 mAb bound the peptope with ~ 0.5 - 3 nM affinity. In the absence of peptope, 7D8 did not bind Aβ(1-40), IAAP fibrils, or ATTR amyloid extracts; however immunoreactivity (~5 nM EC50) was observed following peptope pre-treatment. The mAb 7D8 did not bind ATTR amyloid in FFPE tissue sections when applied alone or in the presence of the control peptide (p5; Fig. 1). However, when the tissue was pretreated with peptope there was dramatic and specific reactivity with the amyloid (Fig. 1). Finally, when opsonized with peptope and mAb 7D8, ATTR human amyloid extracts were efficiently phagocytosed by cultured RAW 246.7 macrophages.

DISCUSSION: Based on these promising data we therefore hypothesize that by using the peptope technology it may be possible to extend the immunoreactivity and utility of mAbs, such as 7D8, for use in patients with ATTR and other amyloid deposits that naturally lack direct reactivity to the antibody. The combination of the peptope with 7D8 may provide a novel pre-targeting system for pan-amyloid immunotherapy.

Fig. 1: Immunostaining of ATTR with mAb 7D8 in the absence (left) or presence (right) of the peptope. Original mag. 160x.
Acceleration of α-synuclein aggregation

R Gaspar¹, M Grey¹, C Dunning², G Meisl³, A Buell⁴, TJP Knowles³, S Linse² and E Sparr¹

¹Department of Physical-Chemistry, ²Department of Biochemistry and Structural Biology, Lund University, Lund, Sweden. ³Department of Chemistry, University of Cambridge, Cambridge, UK. ⁴Institute of Physical Biology, University of Düsseldorf, Düsseldorf, Germany.

Ricardo.Gaspar@fkem1.lu.se

INTRODUCTION: We have recently reported that the underlying aggregation mechanism of α-synuclein (α-syn) is dominated by an autocatalytic secondary process at mildly acidic pH [1], solution conditions mimicking those in endosomes and other specific organelles. Recent experiments were performed to identify the nature of these secondary processes, distinguishing between fragmentation of fibrils and nucleation of monomer on the surface of existing aggregates.

In amyloid plaques, associated with several amyloidogenic diseases, tightly associated lipids have been identified. We explore how lipid membranes interfere with the aggregation of α-syn for different lipid systems [2], and investigate lipid-protein co-aggregation.

MATERIAL & METHODS: We use recombinant α-syn purified using heat treatment, ion exchange and gel chromatography [2]. Exosomes were isolated from neuroblastoma cells [2]. Lipids were purchased from Avanti Polar Lipids (DOPC, DOPS and Gangliosides). Thioflavin-T kinetics, Cryo-TEM and MS analysis were performed as described in reference 2. Differential sedimentation was used to investigate size distribution profiles of α-syn aggregates relying on the fact that aggregates of different sizes travel through a sucrose gradient at different speeds. QCM-D was performed as described in reference 4. Lipid quantification of the co-aggregates was achieved using a phosphorus assay.

RESULTS: Using differential sedimentation to analyse fibril size distribution, no spontaneous fragmentation of fibrils was observed. Incubating monomeric α-syn for short time periods with trapped fibrils, followed by filtration lead to acceleration of aggregation due to the formation of small oligomeric species. These results clearly suggest secondary nucleation of monomers on the fibril surface. We also investigated the association and dissociation of monomers to pre-formed fibrils which revealed pH dependent, using QCM-D.

A clear dependence of the aggregation rate on lipid composition and lipid charge was found. Exosomes, small vesicles isolated from neuroblastoma cells, were shown to enhance α-syn aggregation, and this catalytic effect was found to be due to the neuro-specific lipid component Gangliosides. Finally, analysis of the mature amyloid fibrils formed in the presence of lipid membranes revealed uptake of lipids into co-aggregates, as well as, fibril-associated vesicles (Fig 1). Identification and a qualitative approximation to lipid composition of the co-aggregates were accessed by phosphorus assays, pointing again towards a selective lipid uptake upon co-aggregation.

DISCUSSION & CONCLUSIONS: The experiments suggest that at mildly acidic pH, the dominant source of new amyloid fibrils is the formation of new nuclei on the surfaces of existing fibrils, hence rationalizing the autocatalytic nature of the process.

Lipid-protein interaction is a highly relevant aspect as some lipid classes have the ability to trigger α-syn aggregation, enhancing the rate of primary nucleation [2, 3].


Fig 1: Cryo-Tem image of α-syn co-incubated with 9:1 DOPC:GM1 vesicles taken after the aggregation process was completed.
CARBAMYLATION OF THE AMINO-TERMINAL RESIDUE (GLY 1) OF MOUSE SAA1.1 PROMOTES AMYLOID FIBRIL FORMATION

B Kluve-Beckerman1, JJ Liepnieks1, MD Benson1,2, X Lai3, G Qi3, M Wang3

1 Department of Pathology and Laboratory Medicine, Indiana University School of Medicine,
2 Roudebush VA Medical Center; 3Indiana University School of Medicine Proteomics Core, Indianapolis, Indiana, USA
bkluvebe@iupui.edu

INTRODUCTION: Many people experience elevated levels of serum amyloid A (SAA) due to chronic inflammation, but only a small percentage develop reactive (AA) amyloidosis. To investigate determinants of the SAA → AA pathogenic pathway, we utilize a cell culture system in which recombinant mouse SAA1.1 undergoes conversion to Congo red-positive fibrils. While most preparations of SAA form fibrils, some are resistant. The goal of this study was to determine the biochemical basis for differences in amyloid-forming proclivity. It is known that fibril formation requires and likely initiates in the amino-terminal region of SAA. Consistent with the importance of this region, our current data show the amino-terminal residue of SAA is carbamylated in preparations which exhibit fibril formation and unmodified in those failing to form fibrils.

METHODS: Mouse SAA1.1 was produced in E. coli and purified under denaturing conditions. The purified protein was characterized by SDS-PAGE, western analysis, amino acid sequencing (Edman degradation), and liquid chromatography mass spectrometry (LC/MS/MS) using C8 and C18 HPLC for fractionation of tryptic peptides. The amyloid fibril-forming capacity of SAA was tested in peripheral blood mononuclear cell (PBMC) cultures supplemented with SAA (160 µg/ml). After 8 days, cultures were stained with Congo red to identify amyloid deposits. Protease sensitivity was compared by incubating SAA preparations at 37⁰C and evaluating by western analysis relative amounts of intact SAA and SAA cleavage fragments in media at selected time points.

RESULTS: The first step in biochemical characterization of SAA was fractionation by chromatofocusing which yielded two peaks differing in pI by 0.1 unit. Both peaks comprised SAA in pure form. Peak 1 did not produce amyloid fibrils in PBMC cultures and was determined by amino-terminal sequencing to have an unmodified amino-terminus (Gly1). In contrast, Peak 2 formed fibrils and had a blocked amino-terminus, suggesting modification of the alpha amino group of residue 1 (Gly1). Tandem mass spectrometry, performed to identify and locate post-translational modifications, revealed that Gly1 in amyloid-forming preparations was carbamylated. While lysine residues throughout SAA were also carbamylated to varying degrees, carbamylation of Gly1 was the modification that showed positive correlation with amyloid formation. Peak 1 (unmodified Gly1, amyloid-negative) was then incubated under carbamylating conditions. This treatment resulted in modification of Gly1 and acquisition of amyloid-forming capacity. Toward understanding how carbamylation might facilitate amyloid formation, Peak 1 and Peak 2 were compared in terms of susceptibility to proteolytic cleavage. Cleavage of intact amyloid-forming, carbamylated Peak 2 SAA into amino-terminally truncated fragments was delayed relative to the cleavage of amyloid-resistant, non-carbamylated Peak 1 SAA.

DISCUSSION: Prior to performing the analyses described above, all chromatofocusing fractions which contained SAA in pure form were pooled to maximize yield. While preparations appeared pure, we now know from mass spectrometric data that they can be heterogeneous with respect to sites and extent of carbamylation (C), and that this modification (specifically C-Gly1) is a determinant of fibril formation in PBMC cultures. In vivo, amino groups on proteins become carbamylated by reacting with isocyanate generated by decomposition of urea or by neutrophil myeloperoxidase-catalyzed conversion of thiocyanate. Although carbamylation of recombinant SAA1.1 is an artifact of purification in 6 M urea, the association of this modification with amyloid fibril formation invites the hypothesis that carbamylation of SAA in vivo may have the same amyloid-enabling effect. This seems especially credible considering the physiological states during which carbamylation is most likely to occur, namely inflammation, uremia, and aging. We propose that SAA when present at high levels and for sustained periods is vulnerable to carbamylation, and that C-Gly1 in particular may slow proteolytic cleavage in the amino-terminal portion of SAA and thus help preserve the region known to initiate fibril formation.
The Kinetics and Mechanism of Amyloid Formation

Christopher M Dobson

Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK

ABSTRACT

Interest in amyloid formation by peptides and proteins has developed with extraordinary rapidity in recent years, primarily because of the links between this phenomenon and a range of debilitating medical disorders ranging from systemic amyloidosis to Alzheimer’s disease. Recent progress in understanding the factors affecting the stability of the amyloid state relative to that of the native state of a protein, along with the development of methods for defining the kinetics and mechanism of the conversion between the different states, has led to a much more detailed understanding of the links between protein aggregation, amyloid formation and human disease. This talk will give an overview of recent advances in this field of study and discuss recent progress from our own laboratory towards understanding the structural and physical properties of the amyloid state, the kinetics and mechanism of its formation, and the nature and origins of its links with disease. In addition, the talk will discuss the ways in which protein aggregation and amyloid formation may be inhibited or suppressed, both to understand the nature of protein aggregation in normally functioning organisms and also to address the development of therapeutic strategies through which to combat the loss of homeostasis and the onset and progression of disease.

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Co-fibrillogenesis of wild-type and D76N β2-microglobulin: seeding effect or prion-like mechanism?

S Raimondi1, A Natalello2,3, S Giorgetti1, G Verona1,4, L Marchese1, R Porcari4, G Faravelli1, A Gallanti1, A Relini5, I Zorzoli6, D Ami2,3, M Valli1, PP Mangione1,4, SM Doglia2,3, V Bellotti1,4

1 Department of Molecular Medicine, University of Pavia, Pavia, Italy. 2 Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy. 3 Consorzio Nazionale Interuniversitario per le Scienze Fisiche della Materia, Unit of Milano-Bicocca, Milan, Italy. 4 Centre for Amyloidosis and Acute Phase Proteins, University College London, London, UK. 5 Department of Physics, University of Genoa, Genoa, Italy. 6 Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy.

sara.raimondi@unipv.it

INTRODUCTION: Wild type β2-microglobulin (β2m) is the amyloidogenic precursor of fibrils deposited in bones and joints in dialysis related amyloidosis (DRA). Its structure, function and role in amyloidogenesis have been widely studied [1]. The first natural amyloidogenic variant of β2m (D76N β2m) [2] is thermodynamically unstable and rapidly forms amyloid fibrils in vitro under fluid agitation of physiological buffer and exposure to hydrophobic surface [3]. The mechanism of intra and inter individual amyloid propagation is under extensive debate and the elucidation of this mechanism can provide crucial clues for interpreting the natural history of the disease.

MATERIAL & METHODS: Time course of aggregation of recombinant wild type and D76N β2m in the presence and in the absence of α-crystallin was carried out under physiological conditions of pH, ionic strength and temperature and monitored by thioflavin T fluorescence emission, electrophoretic analysis of the soluble fraction and electron microscopy. A combination of Fourier Transform Infrared spectroscopy in attenuated total reflection and atomic force microscopy was used to monitor the protein conformational changes occurring during aggregation. Fibril stability was also determined by assessment of the free monomer after denaturation of fibrils.

DISCUSSION & CONCLUSIONS: We have applied our in vitro model of fibrillogenesis to study the amyloid propagation of D76N β2m variant and its effect in the amyloidogenic transformation of wild type β2m. Our data suggest that a nucleation mechanism can properly explain the natural tendency of D76N β2m variant to form fibrils. We exclude a prion like effect and we highlight the role of chaperones in protecting the amyloidogenic transformation of wild type protein [4].

REFERENCES:

Molecular determinants of IAPP cross-amyloid interaction with Aβ

M Bakou, K Hille, M Kracklauer, Anna Spanopoulou, Li-Mei Yan, Andrea Caporale,

A Kapurniotu

Division of Peptide Biochemistry, Technische Universität München, Freising, Germany.

akapurniotu@wzw.tum.de

INTRODUCTION:

Amyloid self-assembly underlies numerous devastating diseases including Alzheimer’s disease (AD) and type 2 diabetes (T2D). Cross-amyloid interactions are able to modulate amyloid self-assembly. However, the molecular mechanisms underlying their effects have not yet been understood. Increasing amounts of evidence suggest that onset and pathogenesis of AD and T2D are linked to each other. The interaction between their key amyloid polypeptides, β-amyloid peptide (Aβ/AD) and islet amyloid polypeptide (IAPP/T2D), could be a possible molecular link. In fact, seed amounts of Abeta fibrils have been found to accelerate IAPP amyloidogenesis whereas interaction between soluble non-fibrillar Abeta and IAPP species suppresses amyloidogenesis (1, 2, 3). We have previously shown that IAPP uses the same binding sites (“hot segments”) for both its amyloid self- and its hetero-assembly with Aβ (4). Thus, the IAPP surfaces mediating hetero- and self-assembly may share some similarities; however, differences should also exist. Here we will present results of our studies toward the identification of key molecular determinants of the cross-amyloid interaction of IAPP with Aβ versus its amyloid self-assembly.

MATERIAL & METHODS:

We applied solid phase peptide synthesis methodology to synthesize human IAPP, Aβ and a number of different IAPP mutants and various different biophysical/biochemical methods including circular dichroism and fluorescence spectroscopies, the ThT binding assay, and the MTT reduction assay to characterize peptide interactions, conformations, and amyloidogenic and cell-damaging potentials.

RESULTS:

Our studies uncovered key IAPP residues and molecular determinants of its interaction with Aβ versus its self-assembly and characterized their role in IAPP solubility and amyloidogenic and cell-damaging potentials.

DISCUSSION & CONCLUSIONS:

Our results should contribute to understanding IAPP cross-amyloid interaction with Aβ versus its amyloid self-assembly and thus designing molecules and strategies to control these processes. In addition, our findings should assist in designing IAPP analogs with optimized functional profiles for the treatment of T2D, AD or other diseases. Finally, as similar molecular principles govern interactions between different polypeptides, our findings may apply to other cross-amyloid interactions and related amyloid diseases as well.

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CROSS-SEEDING OF MEDIN AND Aβ AMYLOID

HA Davies1, K Degenhardt2,3, JJ Neher2,3, J Madine1

1Department of Biochemistry, University of Liverpool, Liverpool, U.K. 2Department of Cellular Neurology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany. 3German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany.

h.davies1@liverpool.ac.uk

INTRODUCTION: Aortic medial amyloid is the most common form of localised amyloid and is found in the medial layer of aged aortas1. Our recent work has shown that medin/MFGE8 co-localises with the amyloid plaques of Alzheimer’s disease mouse models (unpublished). To date very little is known about the nature of this interaction and whether it may have a role in disease progression through a seed-like mechanism or through peptide co-aggregation. In our previous work we have highlighted the structural similarities between medin and amyloid-ß (Aß) that could facilitate such interactions2. We aimed to investigate this hypothesis and see if medin and Aß could heterologously seed each other and furthermore to see if they were capable of forming mixed protein fibrils (co-fibrilisation).

MATERIAL & METHODS: Proteins were made recombinantly in E.coli. Seeds were produced from either recombinant amyloid-like fibrils or amyloid fibrils extracted from tissue and sonicated. Fibril formation and kinetic analysis were carried out primarily using thioflavin T fluorescence assay. Fibril morphology and composition were assessed using immunolabelled transmission electron microscopy.

RESULTS: Our results indicated that medin and Aß are capable of cross-seeding each other in vitro. Furthermore, when medin and Aß were co-incubated we observed evidence of co-fibril formation through altered fibril morphology and immunolabelling for both medin and Aß peptides. Interestingly, we observed a difference in seeding efficacy between Aß40 and Aß42.

DISCUSSION & CONCLUSIONS: These results may represent a new and exciting twist in the understanding of amyloid formation and the role of vascular factors in Alzheimer’s disease. Further work needs to be carried out to determine the exact nature of this interaction and more importantly the implications for pathology. Moreover, it is necessary to establish whether this interaction occurs in humans.

REFERENCES:


Fig. 1: Medin cofibrilisation and seeding capabilities. (A) Transmission electron microscopy image of immunolabelled mixed Aß40 and medin fibrils. (B) Pronounced seeding effect of medin seeds on Aß40 aggregation observed by thioflavin T fluorescence.
Immunotherapeutic clearance of systemic amyloid deposits by antibodies to serum amyloid P component

Mark B Pepys

Wolfson Drug Discovery Unit, Centre for Amyloidosis and Acute Phase Proteins,
Royal Free Campus, University College London, UK

The extracellular amyloid deposits which disrupt tissue architecture are unequivocally pathogenic and a major cause of organ dysfunction in all forms of systemic amyloidosis. Reduction of amyloid load is therefore highly desirable. Antibodies to serum amyloid P component (SAP) achieve this objective by targeting the universally present SAP in all human amyloid deposits. The approach is uniquely made possible by the drug CPHPC (hexanoyl bis(D-proline) which safely and potently depletes circulating SAP but leaves some SAP in the amyloid deposits to serve as the antigen target for the therapeutic antibody. Only then is it feasible to administer anti-SAP antibody. Amyloid clearance depends on classical complement pathway activation and macrophages. It is mediated by C3 deposition on the amyloid and formation of multinucleated giant cells which are specifically equipped to surround, engulf and destroy large complement opsonised objects. The obligate therapeutic partnership of CPHPC and anti-SAP antibody has been developed by GlaxoSmithKline and is effective in patients with systemic AL AA, AApoAI and AFib amyloidosis. Amyloid clearance is unequivocally confirmed by reduction of extracellular volume, improvement in SAP scintigraphy and decreased liver stiffness. Anti-SAP triggered increased immediate production of IL-6, but not TNFα. Early acute-phase responses of CRP and SAA were followed by substantial plasma C3 depletion. Variable infusion reaction were abrogated by hydrocortisone and antihistamine premedication but most subjects receiving higher antibody doses developed skin rashes, though only one was serious and it responded to oral prednisone. Importantly, no increased dysfunction was observed in any amyloidotic or other organs, including the kidney. On the contrary, reduction of amyloid load was associated with improved organ function, especially in the liver. Repeat antibody doses progressively reduced amyloid load with corresponding functional improvement. Anti-SAP antibody thus triggers rapid non-tissue damaging amyloid clearance from the extracellular space, a process which is otherwise very slow or absent. A phase 2 trial is now planned of CPHPC plus repeat anti-SAP antibody dosing in patients with cardiac AL and ATTR amyloidosis in whom heart involvement causes major morbidity and mortality.


Regression of cardiac AL amyloidosis demonstrated by cardiovascular magnetic resonance: a new era of understanding

A Martinez-Naharro, DS Knight, TA Treibel, Abdel-Gadir, G Zumbo, S Rosmini, T Lane, S Mahmood, S Sachchithanantham, CJ Whelan, HJ Lachmann, JD Gillmore, AD Wechalekar, JC Moon, PN Hawkins, M Fontana

National Amyloidosis Centre, UCL Division of Medicine, Royal Free Hospital, London, United Kingdom
anamartinez.naharro@nhs.net

INTRODUCTION: The presence and severity of cardiac involvement in immunoglobulin light chain (AL) amyloidosis is the major determinant of survival, along with haematological response to chemotherapy. Cardiac organ responses are currently assessed by tracking serum NT-proBNP and echocardiograms, but neither is able to specifically quantify amyloid burden. The aim of this study was to assess the role of cardiovascular magnetic resonance (CMR) in tracking cardiac response including cardiac amyloid burden in patients with AL amyloidosis undergoing chemotherapy.

MATERIAL & METHODS: 28 patients with biopsy proven cardiac AL amyloidosis were analysed. Patients were assessed with ECG, Echocardiogram, CMR, SAP scintigraphy and NT-proBNP measurements before and after treatment. CMR included volumes, late gadolinium images and T1 mapping for extracellular volume measurement (ECV), which estimates the amyloid burden.

RESULTS: The 28 patients included 18 males (64%), mean age 61 (SD 8.86) years. The interval between assessments was 18±12 months. The overall haematologic response rate was 65% (36% complete response, CR; 29% very good partial response, VGPR). Ten patients (36%) had either a partial response (PR) or no response (NR). Those attaining a CR or VGPR had a significant decrease in amyloid burden and NT-proBNP concentration (median ECV fell from 56% to 43% and median total amyloid volume from 102 to 71g, p<0.05). When patients were analysed according to decrease in amyloid burden, regressors showed a significant improvement in NT-proBNP, LV mass, left atrial area and diastolic function parameters. Regression of cardiac amyloid by CMR correlated with regression of visceral amyloid by SAP scintigraphy.

DISCUSSION & CONCLUSIONS: This is the first demonstration of cardiac amyloid regression by CMR. Furthermore, these pilot data show that regression of amyloid from the heart is a relatively common phenomenon following successful chemotherapy. CMR with T1 mapping is able to quantify the amyloid burden and track treatment response, and has the potential to become an essential routine tool in the routine clinical and research evaluation of patients with cardiac amyloidosis.

REFERENCES:


Fig. 1: Left top; patient on CR with subendocardial LGE before treatment and no LGE after treatment. Left bottom; patient on PR with subtle subendocardial LGE before treatment and established subendocardial LGE after treatment. Right; correlation between first and second studies and NT-proBNP, LV Mass, ECV and total amyloid Volume. There is a significant decrease in NT-proBNP, ECV and total amyloid volume in the follow up scan in patients with CR and VGPR. CI indicates confidence interval.
Visualisation of transthyretin heart amyloidosis by 11C-PIB and PET

B Pilebro1, S Arvidsson2 P Lindqvist3, T Sundström1, P Westermark4, G Antoni5, OB Suhr6, J Sørensen5.

1Heart Centre, Cardiology and 6Medicine, Department of Public Health and Clinical Medicine; 2Heart Centre, Department of Surgical and Perioperative Sciences, Clinical Physiology and 3Department of Radiation Sciences, Diagnostic Radiology, Umeå University, Umeå, and Departments of 4Immunology, Genetics and Pathology and 5Medicinal Chemistry, Uppsala University, Uppsala, Sweden.

INTRODUCTION: A number of different cardiac imaging modalities to diagnose amyloid deposition in the heart have been developed of which 99mTc-DPD scintigraphy has been advocated for imaging cardiac amyloid in ATTR amyloidosis. However, 99mTc-DPD scintigraphy appears to detect ATTR amyloid containing TTR fragments only (1). PET utilising a 11C- labelled thioflavin T derivate, Pittsburgh compound B (11C-PIB), which is the gold standard for imaging brain amyloid in Alzheimer’s disease, and which recently also have been shown to identify both AL and ATTR cardiac amyloidosis (2). We wanted to investigate if 11C-PIB could identify ATTR heart deposits with and without presence of TTR fragments.

MATERIAL & METHODS: Ten patients with biopsy proven V30M ATTR amyloidosis were selected for the investigation according to ATTR fragmentation (Group A: fragmented TTR (n=5), group B: full length TTR (n=5)). All underwent 99mTc-DPD scintigraphy and 11C-PIB PET for detection of amyloid deposition in the heart and echocardiography for assessment of septal end-diastolic wall thickness and left ventricular (LV) global longitudinal strain. A LV 11C-PIB retention index (PIB-RI) was calculated for all patients and compared to that of normal volunteers (n=5). Heart biopsy had confirmed the presence of amyloid in one patient with negative 99mTc-DPD scintigraphy and absence of TTR fragments (type B fibrils.)

RESULTS: PIB-RI was increased in all patients compared to controls (p<0.001), and was significantly higher in group B compared to that of group A (0.129±0.041 vs. 0.040±0.006 min⁻¹, p=0.009). Echocardiography parameters were similar in both patient groups (Table I). No 99mTc- DPD uptake was noted in any patient with type B fibrils.

DISCUSSION AND CONCLUSIONS: Our results indicate that the heart is a targeted organ for amyloid deposition in ATTR amyloidosis. 11C-PIB -PET has the potential to identify cardiac amyloid deposits irrespective of fibril composition, even in patients without cardiac hypertrophy. In this small cohort higher 11C-PIB retention was seen in patients with type B amyloid than in those with type A.


Table 1.

<table>
<thead>
<tr>
<th>ATTR fragments present</th>
<th>Full-length ATTR</th>
<th>P-value</th>
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<tr>
<td>IVSD (mm) range</td>
<td>14 (12-16)</td>
<td>13 (12-14)</td>
</tr>
<tr>
<td>LV global longitudinal strain (range)</td>
<td>-19.2 (15.9-24.0)</td>
<td>-19.2 (11.7-21.9)</td>
</tr>
<tr>
<td>99mTc-DPD uptake n (%)</td>
<td>5 (100 %)</td>
<td>0 (0 %)</td>
</tr>
<tr>
<td>11C-PIB RI (range)</td>
<td>0.04 (0.03-0.05)</td>
<td>0.14 (0.08-0.18)</td>
</tr>
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</table>

Outcome of echocardiographic, 99mTc-DPD scintigraphic and PET 11C-PIB investigations in relationship to TTR fragmentation. IVSD = end-diastolic inter-ventricular septum thickness. LV = Left ventricular. RI = Retention index.
Multicenter experience of planar technetium pyrophosphate cardiac imaging:

Does preferential cardiac uptake predict survival in patients with

ATTR cardiac amyloidosis?

A Castano1, M Haq6, D Narotsky1, J Goldsmith3, RL Weinberg2, R Morgenstern2, T Pozniakoff5, F Ruberg8, JL Berk7, A Dispenzieri6, M Grogan4, G Johnson5, S Bokhari2, and MS Maurer1

1Division of Cardiology, Center for Advanced Cardiac Care, 2Nuclear Cardiology Laboratory, Columbia University Medical Center; 3Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, USA; 4Departments of Cardiovascular Diseases, 5Radiology and Immunology, and 6Hematology, Mayo Clinic, Rochester, USA; 7Departments of Medicine, 8Amyloidosis Center, Boston University School of Medicine, Boston, USA; Section of Cardiovascular Medicine, Yale University School of Medicine, New Haven, USA.

ac3220@cumc.columbia.edu

INTRODUCTION: Transthyretin-related cardiac amyloid (ATTR-CA) is an increasingly recognized cause of heart failure with preserved ejection fraction (HFpEF)1. Technetium pyrophosphate ($^{99m}$Tc-PYP) cardiac imaging noninvasively detects ATTR-CA2, but the accuracy of this technique in a multicenter study and the relationship of $^{99m}$Tc-PYP myocardial uptake with survival are unknown. In a multicenter experience, we examined the association of $^{99m}$Tc-PYP cardiac avidity with survival in patients with TTR amyloid cardiomyopathy.

MATERIAL & METHODS: We conducted a multicenter retrospective cohort study in 229 subjects evaluated for cardiac amyloidosis who underwent $^{99m}$Tc-PYP cardiac scanning at 3 amyloid specialty centers in the United States. Cardiac retention of $^{99m}$Tc-PYP was graded by a semi-quantitative visual score (range: 0 [no uptake] to 3 [uptake greater than bone]) and a quantitative heart-to-contralateral (H/CL) ratio. H/CL was calculated as total counts in a region of interest (ROI) over the heart divided by background counts in an identical size ROI over the contralateral chest.2 Sensitivity and specificity for detecting ATTR-CA were measured from pooled data. Cox proportional hazards modeling was performed to detect association between demographic and clinical predictors and the outcome measure of time to death after $^{99m}$Tc-PYP scan. Kaplan-Meier curves were used to estimate survival in high and low H/CL ratio groups using the log-rank test.

RESULTS: Among the 229 patients pooled from 3 centers who underwent $^{99m}$Tc-PYP scans, we first excluded 58 who lacked standard testing and in whom the diagnosis of cardiac amyloidosis was uncertain. $^{99m}$Tc-PYP scans in 171 subjects (121 ATTR-CA and 50 non-ATTR-CA) demonstrated 91% sensitivity and 96% specificity for detecting ATTR-CA with AUC of 0.960 (95% CI 0.932-0.981) and positive likelihood ratio (LR) 22.8. Univariable and multivariable Cox proportional hazards regression analyses among subjects with ATTR-CA showed H/CL $\geq$1.6 predicted worse survival (HR 3.895, 95% CI 1.150-13.192, $P$=0.0289 and HR 3.836, 95% CI 1.054–13.959, $P$=0.0413, respectively). In Kaplan-Meier analysis over 5-year follow-up, survival was significantly worse if H/CL was $\geq$1.6 vs. <1.6 ($P$=0.0204 log-rank test) (Fig 1.).

DISCUSSION & CONCLUSIONS: In this multicenter experience, $^{99m}$Tc-PYP cardiac scans conferred high sensitivity and specificity for differentiation of ATTR-CA (irrespective of genotype) from AL and non-amyloid HFpEF patients. A H/CL ratio $\geq$1.6 was associated with worse survival among patients with ATTR-CA. $^{99m}$Tc-PYP should be incorporated into diagnostic and prognostic algorithms for amyloid cardiomyopathy.

REFERENCES:


Fig 1. Kaplan-Meier survival analysis in patients with ATTR-CA comparing H/CL ratio <1.6 vs. ≥1.6. Kaplan-Meier survival curves are shown among subjects with ATTR-CA comparing H/CL ratio <1.6 vs. ≥1.6 over the 5-year study duration.
CARDIAC AMYLOID IMAGING WITH 18F-FLOBETABEN POSITRON EMISSION TOMOGRAPHY: A PILOT STUDY

WP Law¹, WYS Wang², PT Moore²,³, P Mollee², ACT Ng²

¹ Department of PET and Molecular Imaging, Princess Alexandra Hospital and The University of Queensland, ² Department of Cardiology, Princess Alexandra Hospital and The University of Queensland, ³ Amyloidosis Centre, Princess Alexandra Hospital and The University of Queensland

peter.mollee@health.qld.gov.au

INTRODUCTION: We aimed to determine the feasibility of 18F-florbetaben positron emission tomography (PET) in diagnosing cardiac amyloidosis, and correlate with biventricular myocardial contractile function.

METHODS and RESULTS: ¹⁸F-florbetaben PET was performed in 14 subjects: 5 AL amyloid, 5 ATTR amyloid, and 4 control subjects with hypertensive heart disease. Qualitative and quantitative assessments of ¹⁸F-florbetaben activity were performed using mean standardized uptake value (SUV) of the left ventricular (LV) myocardium and blood pool, and calculation of target-to-background SUV ratio. Percentage myocardial ¹⁸F-florbetaben retention was also calculated as the percentage mean myocardial SUV change between 0-5 mins and 15-20 mins after radiotracer injection. Global LV longitudinal and right ventricular (RV) free wall longitudinal strain were calculated using 2D speckle tracking echocardiography. Target-to-background SUV ratio and percentage myocardial ¹⁸F-florbetaben retention were higher in amyloid patients compared to hypertensive control subjects. A cut-off value of 40% was able to differentiate between cardiac amyloid patients and hypertensive control subjects (Fig 1). Percentage myocardial ¹⁸F-florbetaben retention was an independent determinant of both global LV longitudinal and RV free wall longitudinal strain via an inverse curve relationship.

CONCLUSIONS: ¹⁸F-florbetaben PET imaging can accurately identify and differentiate between cardiac amyloidosis and hypertensive heart disease. Percentage myocardial ¹⁸F-florbetaben retention was an independent determinant of myocardial dysfunction in cardiac amyloidosis.

Fig 1. Boxplots for percentage myocardial ¹⁸F-florbetaben retention for AL amyloid, ATTR amyloid and hypertensive control subjects. The percentage myocardial ¹⁸F-florbetaben retention was significantly higher in AL/ATTR amyloid patients compared to hypertensive control subjects. All of the cardiac amyloid patients and none of the hypertensive control subjects had a myocardial retention of > 40%.
Over the last 45-years transthyretin amyloidosis has emerged from being a rare familial form of peripheral neuropathy (FAP) to the status of a well-recognized world-wide disease of prominent interest for numerous medical specialties. As with many diseases, scientific breakthrough was occasioned by identification of the causative protein (prealbumin / transthyretin). With knowledge of the amyloid protein, identification and characterization of the TTR gene was soon to follow. Now we have >130 TTR mutations associated with TTR amyloidosis and older people with ATTR wild-type are presenting in increased numbers. Fortunately, specific therapies for ATTR are the goal of several research laboratories and pharmaceutical companies. Stay tuned
O25

Antibody therapy for transthyretin related familial amyloid polyneuropathy-another therapeutic option-
Yukio Ando

Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto Japan

ABSTRACT

Although transthyretin (TTR) related familial amyloid polyneuropathy (FAP) was considered to be an intractable disease, liver transplantation can halt the progression of systemic disorders by the suppression of amyloidogenic TTR production, and TTR stabilizers, such as diflunisal and tafamidis, is effective for neuropathy of the patients at early stage. However, those therapies have several problems. Antibody therapy recognizing a cryptic epitope may suppress the TTR amyloid formation process and brake amyloid deposition.

Although it has been widely accepted that conformational change of the monomeric form of TTR is rate limiting step for amyloid formation and deposition to the organs, no effective therapy targeting this step is available. We first developed antisera for TTR115-124, and confirmed that it only reacted with amyloid fibrils in the tissues, but not with serum TTR of FAP patients. We generated a mouse monoclonal antibody T24, which recognized cryptic epitope of conformational changed TTR. The antibody inhibited non-fibrilar TTR accumulations in gastrointestinal tract of FAP model rats having human ATTR V30M gene. Additionally, humanized T24 (RT24) also inhibited TTR fibrillation, and promoted iPS derived macrophage phagocytosis of aggregated TTR.

Higaki et al. developed another monoclonal antibody whose antigen was TTR89-97. The antibody showed similar effects as described above.

Our antibody doesn’t recognize normal serum TTR which is functioning properly in the blood, so these results demonstrate the antibody would be an effective novel therapeutic antibody for FAP.

REFERENCES:

O28
PREPARATION OF MISFOLDED TTR OLIGOMERS UNDER PHYSIOLOGICAL CONDITIONS IN VITRO AND A PRELIMINARY STRUCTURE–PROTEOTOXICITY RELATIONSHIP STUDY

Yvonne S. Eisele and Jeffery W. Kelly,

The Scripps Research Institute

ABSTRACT
We have developed a reliable approach to make misfolded TTR oligomers of regular structure in vitro under physiological conditions and we have shown that these structures exhibit proteotoxicity in a multi-cell organism. Preliminary electron microscopy images of the misfolded TTR oligomers will be presented.

REFERENCES:
The XV International Symposium on Amyloidosis
Clinical implications of amyloid fibril composition in ATTR-amyloidosis

Ole B Suhr

Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

The findings from the familial amyloidosis world transplant registry (FAP-WTR) clearly show a pronounced difference in survival between early and late onset transthyretin amyloid (ATTR) amyloidosis Val30Met patients, but not between early and late onset non-ATTR Val30Met patients. This remarkable variation in survival after liver transplantation for various groups of hereditary patients has not been fully understood.

The detection of two distinct different types of amyloid fibrils in ATTR amyloidosis patients, and its relationship with the phenotype of the disease, and outcome of diagnostic examinations and of liver transplantation offers an explanation, and indicate that various pathways for amyloid fibril formation may be operating.
Domino liver transplantation: Full-length transthyretin in donor and recipient patients with ATTR Val30Met amyloidosis

Per Westermark, Greg Nowak, Ole B. Suhr, Bo-Göran Ericzon

Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden
Division of Transplantation Surgery, Karolinska University Hospital, Stockholm, Sweden
Department of public Health and Clinical Medicine, Umeå University, Umeå, Sweden

Swedish hereditary Val30Met ATTR amyloidosis occurs in two forms with either only full-length TTR molecules in the fibrils (type B) or with fibrils containing a mixture of full-length and fragmented TTR species (type A). While type B patients have responded well to liver transplantation, type A patients tend to progress in amyloid cardiomyopathy and neuropathy after transplantation due to recruitment of wild-type TTR molecules. Determining factors for the two types are unknown and both liver and peripheral mechanisms are possible. A number of patients who have received a liver transplant from a patient with ATTR amyloidosis (‘domino transplantation’) have developed amyloidosis. Comparing the types (A or B) between donor and recipient may shed light on this question.

MATERIAL & METHODS. Subcutaneous adipose tissue biopsies were obtained from 11 recipients of livers from patients with hereditary Val30Met ATTR amyloidosis. Amyloid type was determined by western blot with the aid of an antiserum which recognizes both full-length and fragmented TTR and compared with the results earlier obtained with the donor amyloid.

RESULTS. Eight of the 11 liver recipients had varying amount of amyloid in fat tissue. Time from liver transplantation was 3-9 years. In 7 of the recipients amyloid was of type B and in 1 of type A. Data concerning amyloid type in the donor patient was available for 6 livers and all were of type B. Importantly, the ATTR type in donor and recipient was identical in all 6 available cases, irrespective of the age of the recipient. Unfortunately, no determination of amyloid fibril composition had been performed in the donor, a 68 year old man to the recipient that developed amyloidosis with type A fibrils.

DISCUSSION. The high frequency of type B fibrils among ATTR liver recipients is remarkable. It is also interesting that donor and recipient fibril type was the same in all studied patients. These findings may indicate that fibril type is determined by liver factors and not by peripheral mechanisms.

<table>
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<tr>
<th>ATTR liver donor</th>
<th>ATTR liver recipient</th>
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<td><strong>Age at Itx</strong></td>
<td><strong>Fibril type</strong></td>
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<tr>
<td>1</td>
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Retinol Binding Protein 4 (RBP4) concentration identifies V122I transthyretin cardiac amyloidosis

M Arvanitis¹, S Simon¹, G Chan², D Fine³, P Beardsley³, M LaValley⁴, D Jacobson², ³, C Koch², JL Berk¹, ², LH Connors², ⁶, FL Ruberg², ³

¹Department of Medicine, Boston University School of Medicine/Boston Medical Center, Boston, MA, USA
²Amyloidosis Center, Boston University School of Medicine/Boston Medical Center, Boston, MA, USA
³Section of Cardiovascular Medicine, Boston University School of Medicine/Boston Medical Center, Boston, MA, USA
⁴Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA
⁵Division of Hematology/Oncology, Department of Medicine, VA Boston Healthcare System, Boston, MA, US
⁶Department of Pathology and Laboratory Medicine, Boston University School of Medicine/Boston Medical Center, Boston, MA, USA

INTRODUCTION: Transthyretin amyloid cardiomyopathy (ATTR) is likely an under-recognized cause of heart failure (HF) in the elderly,¹ owing to misattribution of clinical features to other common co-morbidities such as hypertension. The V122I TTR mutation is carried by 3-4% of African Americans and may represent a significant contributor to HF.² The development of treatments for ATTR warrants identification of new diagnostics and screening tools for these cardiomyopathies. Retinol binding protein 4 (RBP4) is an endogenous ligand that stabilizes TTR and prevents misfolding and aggregation.³ Preliminary data from our Center suggest that RBP4 concentration can differentiate ATTR from other causes of cardiomyopathy, however utility in V122I ATTR remains undefined.

MATERIAL & METHODS: We prospectively recruited and genotyped n = 49 self reported African American patients over age 60 years (33 males) with diagnosis of HF and echocardiographic septal diameter (IVSd) of > 12 mm. Genotype identified n = 47 with wild-type TTR comprising non-amyloid controls and n = 2 patients with newly identified V122I ATTR (prevalence 4.2%). Circulating RBP4, TTR, B-type natriuretic peptide (BNP), and troponin I concentrations, echocardiography, and clinical characteristics were assessed and compared with findings from n = 25 previously identified patients with genotype and biopsy proven V122I ATTR. RBP4 concentration was determined by ELISA. Data were compared by Wilcoxon rank sum test, receiver operating characteristic (ROC) analysis to identify optimal thresholds for V122I ATTR identification, and logistic regression to assess relationships between V122I ATTR and RBP4.

RESULTS: Age, gender and race were not significantly different between V122I ATTR patients and controls. Plasma RBP4 levels were significantly lower in patients with V122I ATTR compared to controls (31.7 µg/mL vs. 49.4 µg/mL, p < 0.001) and the difference persisted after controlling for age, gender, body mass index, cardiac biomarkers and echocardiographic parameters. Troponin I was higher in V122I ATTR (0.27 µg/mL vs. 0.13 µg/mL, p<0.001), while BNP was lower (902 pg/mL vs. 1028 pg/mL, p<0.001), and creatinine was similar (1.47 mg/dl vs. 1.80 mg/dl, p=0.8). ROC analysis identified RBP4 as a potentially sensitive diagnostic of V122I ATTR cardiomyopathy (AUC 0.77). For screening purposes, a cut-off value < 49.5 µg/mL achieved high sensitivity (100% with 95% CI, 100-100%) but low specificity (38% with 95% CI, 26-53%) for V122I ATTR, yielding a negative predictive value (NPV) of 100% in a population with 36% prevalence of the disease. Importantly, 38% of the non-amyloid control cohort had RBP4 values above this threshold.

DISCUSSION & CONCLUSIONS: Circulating RBP4 concentration readily discriminates V122I ATTR cardiomyopathy from non-amyloid HF in an age, gender, and race similar cohort. RBP4 concentration may be useful as a first step in a screening algorithm.


Funding: The study was supported by NIH R21AG050206, R01AG031804, 1UL1TR001430 grants and by the Young Family Amyloid Research Fund.
Establishment of a diagnostic center for amyloidosis in Japan by Kumamoto University

T Yamashita1,2, M Ueda1, M Tasaki1, T Masuda1, Y Misumi1, K Takamatsu1, K Obayashi2, Y Ando1.

1Diagnostic Unit for Amyloidosis, Kumamoto University Hospital, 2Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, 3Department of Morphological and Physiological Science, Graduate School of Health Science, Kumamoto University
taro-yamashita@fc.kuh.kumamoto-u.ac.jp

INTRODUCTION

Although amyloidosis was an intractable disease, recently novel therapies had been applied to several types of amyloidosis. Especially, for hereditary transthyretin (TTR) amyloidosis (familial amyloid polyneuropathy, ATTR-FAP), AL amyloidosis and AA amyloidosis, effective therapies appeared. The purpose of this study was to analyze clinical characteristics of patients with amyloidosis diagnosed by Diagnostic Unit for Amyloidosis, Kumamoto University Hospital, which was established in April 2012 by the support of Kumamoto prefectural government.

PATIENTS & METHODS

This center has been conducting histopathological, genetical, massspectromical, and proteomical analyses as an amyloidosis diagnosis center in Japan. Diagnosis results by Diagnostic Unit for Amyloidosis, Kumamoto University Hospital from Apr 1, 2012 to Feb 29, 2016 were analyzed.

RESULTS

The number of consultations on diagnosis for amyloidosis from all over Japan was 1,595 and increased year by year. Results of type diagnosis were follows; ATTR-FAP: 18.9%, wild-type TTR (ATTRwt) amyloidosis (senile systemic amyloidosis): 8.0%, ALκ: 7.4%, ALλ: 25.5%, AA: 5.3%, iatrogenic (acquired) ATTR: 1.0%, Aβ2M: 1.2%, others (including novel amyloidosis, semenogelin, etc): 4.0%, light chain deposition disease: 0.7%, no TTR gene mutation: 3.1%, no amyloid deposition: 10.3%. Sixty-one cases needed laser microdissection (LMD) and liquid chromatography tandem-mass spectrometry (LC-MS/MS) analysis for diagnosis. Concerning ATTR-FAP, one hundred and twelve patients (including 2 from China and 1 from Brazil) had a diagnosis of ATTR-FAP. Mutations of TTR gene were follows; V30M (p.V50M): 60.3%, V30A, A36D**, G47V*, G47R, S50I, S50R, G53E, L55P*, T59R**, T60A, E61K, K80R**, G83R*, E89K*, I107V, Y114C (*the first cases in Japan, **the first cases in the world). Eight percent of the patients were associated with the endemic area, but whereas 92% of the patients were from non-endemic areas. The age at onset in the endemic area was 38.8 ± 14.8 (35, 26-66) years old and that in non-endemic areas was 59.1 ± 15.8 (65, 22-83) showing significant difference. Thirty three percent of the patients were male in endemic area, meanwhile 92% of the patients was male in non-endemic areas, showing significant difference. It took 2.2 ± 1.5 (2.4, 0.3-4.6) years from onset to diagnose in endemic area, while it took 3.9 ± 2.9 (3.6, 0.1-10.2) years in non-endemic areas showing significant difference. Data are presented as mean ± standard deviation (median, minimum-maximum).

DISCUSSION & CONCLUSIONS

In this study we have demonstrated that there are more numbers of ATTR-FAP patients with V30M or non-V30M mutations in endemic area or non-endemic areas than a number expected before. Our study shows that there are many male, late-onset ATTR-FAP patients in non-endemic areas compared with endemic area. It takes long time to diagnose ATTR-FAP especially in non-endemic areas. For early diagnosis and early intervention, more enlightenment activities and more development of diagnosis systems for ATTR-FAP are needed.
Male gender is a risk factor for myocardial involvement in transthyretin-related amyloidosis: A study based on the Transthyretin Amyloid Outcomes Survey

C Rapezzi1, M Waddington Cruz2, M Lorenzini3, MS Maurer3, AV Kristen4, T Damy5, T Coelho6, C-R Yu7, M-L Ong7, on behalf of THAOS Investigators

1Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Italy. 2Centro de Estudos em Paramiloidose Antônio Rodrigues de Mello, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil. 3Columbia University College of Physicians and Surgeons, New York, NY, USA. 4Amyloidosis Center, Department of Cardiology, Heidelberg University, Heidelberg, Germany. 5Amyloidosis Network, Department of Cardiology, all at CHU Henri Mondor, INSERM U955 and Clinical Investigation Center 006, and DHU A-TVB all at Creteil, Creteil, France. 6Department of Neurology, Hospital de Santo Antônio, Porto, Portugal. 7Pfizer Inc, New York, NY, USA

claudio.rapezzi@unibo.it

INTRODUCTION: Gender-related differences in human amyloidosis is a relatively little explored topic. While no major gender imbalance has been reported for light chain amyloidosis, in transthyretin (TTR) amyloidosis previous small studies of patients with a wide range of mutations suggest that left ventricular (LV) wall thicknesses is greater in men and that wtTTR appears to be essentially a disease of elderly men. We hypothesized that some biological characteristics protect women against myocardial involvement in TTR amyloidosis and/or increase the risk of cardiac involvement in men. We explored this hypothesis within the largest available database on TTR amyloidosis, the ongoing Transthyretin Amyloid Outcomes Survey (THAOS) international registry.

MATERIAL & METHODS: Gender differences between subgroups with different mutations and different organ involvement were explored (cut-off date: January 14, 2016). Group comparisons were performed using one-way analysis of variance. Chi-square analysis was used for nominal data. Multiple linear regression analyses were performed using increasing mean LV wall thickness and LV indexed mass as the dependent variables in order to study associations with gender, age at first observation, and genotype. Symptomatic patients with variant TTR (n=1802), asymptomatic TTR mutation carriers (n=661) and wtTTR (n=363) were studied. Measurements of mean LV wall thickness and LV indexed mass were available for 825 and 734 patients, respectively.

RESULTS: Male prevalence was 56.9% in the entire registry, 47.1% in V30M, 59.1% in nonV30M non cardiac mutations, 70.5% in nonV30M cardiac and 95.0% in wtTTR. Cardiac phenotype and abnormal ECG were both more frequent in males (30.0% vs 8.3%, 63.1% vs 36.3% respectively, p<0.0001). There was no gender imbalance in kidney or sensory abnormalities. Autonomic impairment was more frequent in males (45.8% vs 39.5%, p=0.0009). 83.2% of the 537 patients with an echocardiographically defined cardiomyopathy (CMP) (mean LV thickness>12 mm) were male. LV ejection fraction was higher in females than in men (62.5 vs 55.7, P<0.0001) whereas LV mean wall thickness was lower (15.3 vs 17.0 mm, P<0.0001). On the contrary, neurosensory impairment was more relevant in females with CMP. Males were more prevalent (p<0.0001) among the higher quartiles of increasing LV wall thickness, LV mass (both indexed by height), Karnofsky Performance Status Scale, NYHA Classification, and NT-pro BNP, whereas modified body mass index was not influenced by gender. Male gender, age at presentation, and type of mutation were significantly associated with increasing mean LV thickness and increasing LV mass index (p<0.0001).

DISCUSSION & CONCLUSIONS: In TTR amyloidosis, myocardial involvement is more frequent and pronounced in men, supporting the hypothesis that some biologic characteristics may prevent myocardial amyloid infiltration in women (or facilitate it in men). Further investigations could identify possible underlying protective mechanism, clarify the relationship with the roles of genotype and age, and orient the search for innovative therapeutic approaches.
Unveiling transthyretin cardiac amyloidosis as an etiology for paradoxical low-flow severe aortic stenosis in patients undergoing transcatheter aortic valve replacement

A Castano1, D Narotsky1, R Morgenstern1, N Hamid2, O Khalique2, S Kodali2, RT Hahn2, S Bokhari3, MS Maurer1

1Division of Cardiology, Center for Advanced Cardiac Care, 2Center for Interventional Vascular Therapy, 3Nuclear Cardiology Laboratory, Columbia University Medical Center, New York, USA.

INTRODUCTION: Transthyretin cardiac amyloidosis (ATTR-CA) is a prevalent and important cause of heart failure with preserved ejection fraction in older adults and may be an etiology of paradoxical low flow (PLF) severe aortic stenosis (AS). The prevalence of ATTR-CA among patients with severe AS and its associated factors is unknown but could have implications for valve replacement and long term outcomes. We used technetium pyrophosphate ($^{99m}$Tc-PYP) cardiac imaging to (1) identify ATTR-CA and its prevalence in patients with severe AS undergoing transcatheter aortic valve replacement (TAVR) and (2) identify parameters which may detect ATTR-CA in this population.

MATERIAL & METHODS: 118 patients with severe AS underwent $^{99m}$Tc-PYP cardiac scans within 30 days of TAVR. ATTR-CA was defined as diffuse $^{99m}$Tc-PYP uptake and heart-contralateral (H/CL) ratio $\geq$1.5 (Fig. 1A).2 From transthoracic echocardiography, we performed the following measures previously associated with ATTR-CA: diastolic function (E/A and E/e’ ratio), systolic function (tissue Doppler S’, strain), and myocardial contraction fraction (MCF), a novel measure of myocardial shortening calculated as the ratio of stroke volume (SV) to myocardial volume.3 Tissue Doppler S’ was averaged from the lateral and septal mitral annulus and relative apical longitudinal strain (LS) was defined as average apical LS / (average basal LS + mid LS).4 Demographic and clinical values were analyzed using standard comparative statistics. We compared area under the curve (AUC) for ATTR-CA predictors.

RESULTS: Among 118 pts (mean age 84±6 yrs), $^{99m}$Tc-PYP scan positivity for ATTR-CA was 14% (n=17). Compared to pts without disease, ATTR-CA pts were more likely to be men (80% vs. 61%, p=0.049), with thicker interventricular septum (1.3 vs 1.1 cm, p=0.046), higher left ventricular mass index (127 vs 96 g/m², p=0.005), lower SV index (27 vs 37 ml/m², p=0.023) and greater incidence of PLF (41% vs 23%, p=0.046). The odds ratio for ATTR-CA among patients with vs. without PLF was 2.1. ATTR-CA pts had a higher E/A ratio (1.7 vs 0.9, p=0.02), lower deceleration time (180 vs 257, p=0.0004), and similar E/e’ ratio (19 vs 16, p=0.21). ATTR-CA pts also had lower LVEF (50% vs 60%, p=0.036), avg S’ (4.0 vs 6.5 cm/s, p<0.0001), and MCF (29 vs 41, p=0.0003). Relative apical LS was not different in ATTR-CA positive vs negative patients (p=0.98) but global LS trended lower (p=0.09). Prediction of ATTR-CA was best using avg tissue Doppler S’ (AUC 0.95, 95%CI 0.90-0.99) (Fig. 1B).

DISCUSSION & CONCLUSIONS: ATTR-CA is prevalent among patients with severe AS undergoing TAVR and is frequently associated with PLF. The etiology of PLF may be related to both advanced diastolic dysfunction and reduced myocardial mechanics. ATTR-CA can be accurately predicted echocardiographically by average tissue Doppler S’.

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Fig. 1: Predictors of ATTR-CA Diagnosed by $^{99m}$Tc-PYP Cardiac Scanning. Quantitative assessment of $^{99m}$Tc-PYP myocardial uptake (A) is shown in an ATTR negative (Top) and positive patient (Bottom) with corresponding H/CL ratio calculation. ROC curves for predictors of a positive $^{99m}$Tc-PYP scan are shown (B). AUC, area under the curve; DT, deceleration time; H/CL, heart-to-contralateral ratio; MCF, myocardial contraction fraction; ROC, receiver operating curve; VMR, voltage-mass ratio.
LIMITED DURATION TREATMENT WITH BORTEZOMIB AND METHYLPREDNISONE IS EFFECTIVE IN PRIMARY AMYLOIDOSIS LEADING TO RESULTS SIMILAR TO THOSE OF HIGH DOSE MELPHALAN

Rafat Abonour and Merrill Benson

Indiana University School of Medicine

Systemic amyloidosis (AL) can be put into hematologic and organ remission by halting monoclonal light chain production. High dose melphalan with hematopoietic cell support is standard induction therapy for eligible patients, resulting in an improved median survival to 4-5 years, a hematological response of 30–70% and treatment-related mortality (TRM) as high as 22%. Standard dose melphalan with dexamethasone in patients ineligible for high dose therapy has not shown matched long-term survival outcomes to high-dose therapy. Bortezomib in the relapsed/refractory setting can lead to hematologic response in large number of patients. Palladini et al matched 87 patients ineligible for HDT to receive melphalan, dexamethasone and bortezomib (MBDex) to 87 historic controls of melphalan and dexamethasone (MDex). A complete response was observed in 36 (42%) vs. 16 (19%) of MBDex v MDex group respectively (P = 0.002), and overall response was 69% vs. 51%; however there was no difference in overall survival. We conducted a retrospective, single institution evaluation of 86 patients with AL, comparing high dose melphalan with autologous hematopoietic cell rescue to non-intensive therapy. Non-intensive regimen was bortezomib 1.3 mg/m² IVP on day 1, 8 and 15. Methylprednisone 1000 mg IV was give on day 1, 8 and 15 of 28 days cycles. Patients received on average 4 cycles of therapy. Median DFS was 53 months for patients receiving high dose melphalan and 59 months for those receiving non-intensive dose therapy (P = 0.86). Hematologic remission was seen in 68% of patients and organ remission was seen in 58% of the patients treated with bortezomib. Details disease state and organ involvements will be presented at the meeting. Time to hematologic and organ responses will be also presented at the meeting.

We conclude that treatment to hematologic remission is feasible with limited number of cycles of chemotherapy. Durable organ response was also achieved with this patients’ friendly and cost effective approach. Future randomized clinical trial should include treatment strategies to complete hematologic remission as treatment to progression can lead to excessive toxicities and unnecessary resource utilizations.
Renal outcome among patients with systemic AL amyloidosis who present with advanced CKD is dependent on speed and magnitude of response to chemotherapy.

T Rezk, AD Wechalekar, HJ Lachmann, JA Gilbertson, D Rowzcenio, J Pinney, CJ Whelan, S Mahmood, S Sachchithanantham, T Lane, M Fontana, PN Hawkins, JD Gillmore

National Amyloidosis Centre, Centre for Amyloidosis and Acute Phase Proteins, Division of Medicine, Royal Free Campus, University College London, UK.

t.rezk@ucl.ac.uk

Introduction: Renal amyloid is present in ~70% of patients with systemic AL amyloidosis at diagnosis and manifests with progressive proteinuric chronic kidney disease (CKD) (1). Cardiac amyloid, the main determinant of patient survival, is present in ~50% at diagnosis(2). Chemotherapy is associated with improved patient survival, but its effect on outcomes among patients who present with established advanced CKD remains unknown.

Materials and Methods: 1000 patients with systemic AL amyloidosis were prospectively enrolled into the UK AL amyloidosis chemotherapy (ALCHEMY) study from September 2009 to 2015; 670 had renal involvement of whom 84 had an eGFR <20 ml/min at diagnosis. Of those 84, 39 had co-existing cardiac amyloidosis and 45 had renal amyloidosis without cardiac involvement. We report time from baseline to the composite endpoint of either death or dialysis in relation to response to chemotherapy among all 84 patients with an eGFR of <20 ml/min at diagnosis, and time to dialysis alone (renal survival) in relation to response to chemotherapy among the subset of 45 patients with ‘isolated’ renal amyloid. Response to chemotherapy was defined as percentage reduction in pre-treatment amyloidogenic free light chain (dFLC) concentration, assessed at 1, 3, 6 and 12 months from baseline.

Results: Median age at baseline was 68 yrs and median eGFR was 10 ml/min. Median time to death or dialysis among those who achieved a dFLC response of ≥90% within 3 months of baseline was 22.9 months compared to 5.3 months among patients who achieved lesser degrees of clonal response (p=0.0001) (A). In those with isolated renal amyloid, median renal survival among 12 patients who achieved a dFLC response of ≥90% within 3 months of baseline was 22.9 months compared to 6.1 months among 28 patients who achieved lesser degrees of clonal response (p<0.008) (B). Among 10 patients with isolated renal amyloid who achieved a ≥90% dFLC response after >12 months, renal survival was 7.3 months (p<0.05).

Conclusion: Outcome in patients with systemic AL amyloidosis and advanced CKD is strongly dependent upon the magnitude and speed of clonal response to chemotherapy. Patients with advanced CKD due to renal AL amyloidosis should be rapidly treated with chemotherapy, in order to delay or avert dialysis.


CS1 chimeric antigen receptor adoptive T cell therapy for systemic light chain amyloidosis

X. Wang\textsuperscript{1}, M. Rosenzweig\textsuperscript{1,2}, R. Urak\textsuperscript{1}, M. Walter\textsuperscript{1}, L. Lim\textsuperscript{1}, N. Nathwani\textsuperscript{1,2}, M. Htut\textsuperscript{1,2}, C. Karanes\textsuperscript{1,2}, G. Somlo\textsuperscript{1,2}, A. Krishnan\textsuperscript{1,2}, S. Forman\textsuperscript{1,2}

Department of Hematology and Hematopoietic Cell Transplantation, City of Hope National Cancer Center, Duarte, California, U.S.A \textsuperscript{2}Judy and Bernard Briskin Myeloma Center.

xiuwang@coh.org

INTRODUCTION: The goal of therapy for light chain amyloidosis (AL) is eradication of the abnormal plasma cell clone. Conventional chemotherapy and autologous stem cell transplantation are effective, but new treatments are needed. Chimeric Antigen Receptors (CARs) are emerging as a powerful tool to redirect T cell specificity against cancer. CARs are artificial molecules constituted by an extracellular antigen binding domain consisting of the variable chains of a monoclonal antibody, linked together as a single chain Fv(scFv), and an intracellular signaling region, usually the zeta chain of the TCR/CD3 complex, that is immediately triggered upon antigen recognition. CS1 is a cell surface glycoprotein of the signaling lymphocyte activation molecule (SLAM) receptor family that is highly and selectively expressed on normal plasma cells and multiple myeloma (MM) cells, with lower expression on NK cells and little or no expression on other normal tissue. We and others have shown that plasma cells in AL express CS1 \cite{1}.

MATERIALS & METHODS: To explore the utility of CS1 as a target for CAR T cell therapy for AL amyloidosis, we constructed a second generation CS1 CAR, containing a CD28/41BB costimulatory domain, the ribosomal-skip T2A sequence, and the truncated EGF receptor sequence (EGFR\textsubscript{t}) as a selection, tracking, and ablation molecule and incorporated into a SIN lentiviral vector. Purified central memory T cells were activated and transduced with a lentiviral vector encoding CS1 CAR and expanded in the presence of IL2 50U/ml. Cytotoxicity of the expanded CS1 CAR T cells was evaluated using 4-hour \textsuperscript{51}Cr release assays after co-culture with \textsuperscript{51}Cr-labeled CS1+ target cells (MM.1S). To test the antitumor activity, we inoculated MM.1S into NSG mice by intra-tibial injection. Once the tumor engraftment was confirmed, 1x10\textsuperscript{6} CS1 CAR T cells were infused into tumor-bearing mice intravenously.

RESULTS: The CS1 CAR T cells exhibit specific and efficient killing of CS1 positive cells (MM.1S) (Figure 1). Anti-tumor studies in the animal model showed that CS1 CAR T cells induced significant tumor remission and prolonged survival as compared to mock T cell treated mice.

DISCUSSION & CONCLUSIONS: CS1 CAR T cell therapy opens a new window for eradicating the malignant cells and stopping light chain synthesis by the clonal plasma cells in AL. This strategy extends innovative CAR technology to the treatment of patients with AL amyloidosis.

Figure 1: Specific cytolytic function of CS-1 CAR T cells against CS1 positive tumor cells

References:

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NEOD001 DEMONSTRATES ORGAN BIOMARKER RESPONSES IN PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS AND PERSISTENT ORGAN DYSFUNCTION: RESULTS FROM THE EXPANSION PHASE OF A PHASE 1/2 STUDY

M Liedtke,1 RL Comenzo,2 H Landau,3 V Sanchorawala,4 B Weiss,5 J Zonder,6 J Walling,7 GG Kinney,8 M Koller,8 DB Schenk,8 SD Guthrie,8 E Liu,8 MA Gertz9

1 Stanford University School of Medicine, Stanford, CA, USA. 2 Tufts Medical Center, Boston, MA, USA. 3 Memorial Sloan Kettering Cancer Center, New York, NY, USA. 4 Boston University School of Medicine, Boston, MA, USA. 5 University of Pennsylvania, Philadelphia, PA, USA. 6 Karmanos Cancer Institute, Detroit, MI, USA. 7 JW Consulting, Hillsborough, CA, USA. 8 Prothena Biosciences Inc, South San Francisco, CA, USA. 9 Mayo Clinic, Rochester, MN, USA.
gertz.morie@mayo.edu

INTRODUCTION: Current therapies used to treat AL amyloidosis limit light chain (LC) production but do not directly target deposits underlying multiorgan failure. NEOD001, a monoclonal antibody, targets misfolded LC and is thought to neutralize circulating LC aggregates and to clear insoluble deposits. In an interim analysis of a phase 1/2 dose-escalation study in 27 patients with AL amyloidosis and persistent organ dysfunction (NCT01707264; EudraCT2012-002683-27), monthly infusions of NEOD001 were safe, well tolerated, and associated with renal and cardiac responses.1 Here we report updated results from the escalation phase and new results from the expansion phase of this study.

MATERIAL & METHODS: Patients who completed ≥1 anti–plasma cell systemic therapy, had partial hematologic response or better to any previous therapy, and had persistent organ dysfunction received NEOD001 intravenously every 28 days. During the dose-escalation phase, 27 patients received NEOD001 at 0.5, 1, 2, 4, 8, 16, or 24 mg/kg in a 3+3 study design. An additional 42 patients with renal, cardiac, or nerve involvement were enrolled and treated (24 mg/kg) in the expansion phase. We assessed safety/tolerability, pharmacokinetics, immunogenicity, cardiac and renal responses based on consensus criteria, and neuropathy responses using the Neuropathy Impairment Score-Lower Limbs (NIS-LL).

RESULTS: Twenty-seven patients (median age, 60 years; cardiac involvement, 66.7%) were enrolled in the dose-escalation study. Of 15 renal-evaluable patients, 9 (60%) met criteria for renal response. Of 14 cardiac-evaluable patients, 8 (57%) met criteria for cardiac response. An additional 42 patients were enrolled in the expansion study, which included cohorts with renal (16 patients), cardiac (15 patients), and peripheral nerve (11 patients) involvement. In the overall population, the median age was 60 years, and 60.9% of patients were male. Median (range) time since diagnosis was 3.02 (0.4-16.0) years for the expansion patients and 2.85 (0.4-16.0) years for the overall population. In the expansion phase, the best hematologic response (HR) to the most recent systemic treatment was complete response (CR) in 12 patients (28.6%), very good partial response (VGPR) in 13 patients (31.0%), partial response (PR) in 2 patients (4.8%), no response (NR) in 2 patients (4.8%), and unavailable in 13 patients (31.0%). In the overall population, the best HR to the most recent systemic treatment was CR in 24 patients (34.8%), VGPR in 18 patients (26.1%), PR in 6 patients (8.7%), NR in 4 patients (5.8%), and unavailable in 17 patients (24.6%). Safety/tolerability and organ response data will be presented for the expansion patients.

DISCUSSION & CONCLUSIONS: Our interim results demonstrated that monthly NEOD001 infusions are safe and well tolerated, with organ response rates comparing favorably with traditional chemotherapy. These updated results from the escalation phase and new results from the expansion phase, including results from patients with peripheral nerve involvement, may help to further elucidate our understanding of this therapeutic approach. Antibody therapy may allow for effective treatment of patients with AL amyloidosis and persistent organ dysfunction despite HR or in concert with existing plasma cell–directed therapy as part of initial treatment.

AL AMYLOIDOSIS CAN BE TREATED EFFECTIVELY WITH NON-TRANSPLANT THERAPY

G Merlini, G Palladini

Amyloidosis Research and Treatment Center, Foundation IRCCS Policlinico San Matteo, and Department of Molecular Medicine, University of Pavia, Pavia, Italy
gmerlini@unipv.it

In the last two decades we witnessed a progressive increase of the options available to treat AL amyloidosis, mostly based on advancements made in multiple myeloma. However, differently from multiple myeloma, very few controlled studies have been completed, and novel treatments have entered routine clinical practice without rigorous assessment of their safety and efficacy compared to existing alternatives. In addition, the extreme heterogeneity of AL amyloidosis, with low-risk patients surviving long times even in the absence of response to therapy, whereas high-risk subjects often die before they have a chance to respond, makes it very difficult comparing results of uncontrolled studies.

Substantial treatment-related mortality (TRM) limits the feasibility of autologous stem cell transplantation (ASCT) to a minority of patients with preserved organ function. Response rates (hematologic response ~70%, CR ~35%) and survival (median 7.6 years) are very good in this setting [1, 2], with survival at 10, and 15 years for those with hematologic CR of 72%, and 57%, respectively [2]. Oral melphalan / dexamethasone (MDex) grants similar results (hematologic response 76%, CR ~31%, median survival 7.3 years) with no TRM in intermediate-risk patients [3], although survival at 10 and 15 years of patients achieving CR is not known. Early results of a phase III randomized trial indicate that the addition of bortezomib to MDex increases the response rate (~80%, interim analysis presented at the Symposium). Although these agents cannot change the fate of patients with very advanced cardiac involvement (stage IIIb), combination of alkylating agents (cyclophosphamide or melphalan), bortezomib and dexamethasone, are probably the combinations granting the highest rate of success in this disease [4]. The availability of anti-amyloid strategies that can be used in combination with chemotherapy will likely improve the rate of organ response achievable with non-transplant approaches [5].

So far, there is no convincing evidence in favor of ASCT over non-transplant approaches. Novel controlled trials are warranted, and uncontrolled studies should be reported in a standardized fashion, including rigorous staging and intent-to-treat response, in order to allow fair assessment of outcomes. For now, sequential treatment, with non-transplant chemotherapy, based on patients’ risk profile and preferences and aiming at good quality response, remains the best way to treat the majority of patients with AL amyloidosis.

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In 1994 the Boston Amyloidosis Center pioneered HDM/SCT as frontline treatment for patients with AL amyloidosis. At that time no effective therapy existed for AL amyloidosis and treatment with cyclic oral melphalan (M/P) was the standard of care. M/P prolonged median survival to 12-18 months, however, hematologic and clinical responses as well as long-term survival were rare. A dramatic intervention was needed early in disease before life-threatening organ deterioration in order to have an impact. We proposed HDM/SCT and made the eligibility criteria liberal, in order to be inclusive, and the response criteria strict, in order to be persuasive about efficacy. Now, 22 years later, we review the impact of this treatment.

In 2004 we reported our findings of the first 8 years. More than 50% of 700 patients evaluated were eligible for HDM/SCT; median overall survival had dramatically improved to 4.6 years; for the first time ever improvement in organ dysfunction was seen one year after HDM/SCT; and a hematologic CR, defined as no evidence of plasma cell dyscrasias at 1 year post HDM/SCT was 40%. Treatment-related mortality (TRM) from any cause was 13%, mostly in patients with cardiomyopathy, leading us to be more stringent about eligibility for patients with cardiac involvement. Our next step was to try to improve on the outcomes of HDM/SCT and we tested a role for induction with 2 cycles M/P prior to HDM/SCT vs HDM/SCT alone. This prospective randomized trial showed no improvement in CR and survival disadvantage in patients with cardiac involvement who received induction therapy prior to HDM/SCT. Subsequently, a phase II trial of tandem cycles of HDM/SCT showed an improvement in CR to 67% without an increase in TRM. The next pilot study tested addition of bortezomib in HDM prior to SCT and results were impressive and a successive phase II trial of 2 cycles of induction therapy with bortezomib and dexamethasone followed by HDM/SCT, demonstrated a hematologic VGPR+CR of 77%. Our HDM/SCT data on 629 patients from the last 20 years indicate a TRM of 7.5%, a CR of 40% and median overall survival of 7.63 years.

Hematologic responses were associated with improvement in organ dysfunction and all measures of quality of life (SF-36) following HDM/SCT, especially for those patients who achieved a CR. Furthermore, hematologic CR was not always necessary for prolonged survival; for 195 patients who did not obtain a CR after HDM/SCT 52% achieved an organ response and the OS was 5.9 years. Median renal survival was 13.4 years and 2-year risk of dialysis for stage I, II and III patients with renal involvement was 0%, 3% and 14%.

In summary, we are fortunate that, because of research, clinical trials and the collaboration of investigators, progress over the past 20+ years now allows many treatment options for patients. In deciding frontline treatment, each new therapy should be compared to the durability and efficacy of HDM/SCT. All treatments are best done in Centers with an experienced multidisciplinary team of clinicians to care for very ill patients.

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AA amyloidosis related to familial mediterranean fever: a cohort study of 31 cases.

S Georgin-Lavialle1*, K Stankovic Stojanovic1, A Cez2, V Avellino3, E Hachulla3, A Mekinian4, S Faguer5, T Le Gallou6, J-M Ziza7, M Hamidou8, Z Amoura9, A Hot10, C Louvrier11, I Giurgia11, S Amselem11, J-J Boffa1, D Buob12, G Grateau1,*.

1 Department of internal medicine, Tenon Hospital, Paris, France; 2 Department of nephrology, Tenon Hospital, Paris, France; 3 Department of internal medicine, CHU Lille, Lille, France; 4 Department of internal medicine, St Antoine Hospital, Paris, France; 5 Department of nephrology, CHU Rangueil, Toulouse, France; 6 Department of internal medicine, CHU Rennes, Rennes, France; 7 Department of internal medicine, La Croix St Simon Hospital, Paris, France; 8 Department of internal medicine, CHU Nantes, Nantes, France; 9 Department of internal medicine, Pitié-Salpêtrière Hospital, Paris, France; 10 Department of internal medicine, Hospice Civils de Lyon, Lyon, France; 11 Department of genetic, Trousseau Hospital, Paris, France; 12 Department of pathology, Tenon Hospital, Paris, France; *French adult reference center for AA amyloidosis and autoinflammatory diseases.

Sophie.georgin-lavialle@aphp.fr

INTRODUCTION: Familial Mediterranean Fever (FMF) is a rare but treatable inherited autoinflammatory condition associated with mutations in MEFV. Daily treatment by colchicine can in most cases prevent the occurrence of febrile crisis and chronic peripheral inflammation. Without treatment, some patients develop AA amyloidosis with consequent renal failure and death. Our objective was to describe the main features of FMF-associated AA amyloidosis and the efficacy of interleukin-1 inhibitors in this complication.

MATERIAL & METHODS: We retrospectively analysed all current French FMF-associated amyloidosis cases through the French network for rare diseases.

RESULTS: Thirty french patients were identified (17 women/14 men) including patients from various origins such as Sefarad Jewish (n=15), Armenians (n=7), Arabic (n=4), Turkish (n=2), Georgian (n=1), Russian (n=1) and Libanese (n=1); three patients were from consanguinous families. The median age on study was 50 years old (ranging from 30 to 79). Median age at the diagnosis of amyloidosis was 40 years (ranging from 24 to 69); the median delay of follow up after the diagnosis of amyloidosis was 11 years (ranging from 3 to 30) and on our cohort, only one (3.3 %) patient died. Among the 31 patients, the MEFV gene was sequenced in 19 patients (61.3%), all patients displayed 2 pathogenic mutations in exon 10, and 12 were homozygous for the MEFV M694V mutation. The median delay between the diagnosis of FMF and the diagnosis of AA amyloidosis was 4 years (ranging from 0 to 54). For 11 patients the diagnosis was made concomitantly (35.5%). As for the patients with a previous diagnosis of FMF, two of them recognised that they did not take colchicine. For others, the daily colchicine dose was not sufficient to control peripheral inflammation. All patients displayed renal involvement with proteinuria; for eight patients, the diagnosis was made on salivary gland biopsy (25.8%); 4 had digestive amyloidosis proven on digestive biopsies. One patient had proven cardiac involvement and 3 patients presented with thyroid goitre. Seventeen patients received daily colchicine treatment with a median dose of 1 mg/day. Ten patients were treated by anakinra, an interleukin-1 inhibitor (32.2%), leading to stabilization of renal function in all cases except one. In one patient (3.3%), anakinra was replaced by canakinumab, another interleukin-1 inhibitor. Ten patients (32.2%) underwent kidney transplantation with only one failure; one patient was transplanted 19 years ago with good efficacy until now. Fourteen patients (45%) required dialysis.

DISCUSSION & CONCLUSIONS: AA amyloidosis is a rare but severe complication of FMF which can still occur in 2016 and even lead to a late diagnosis of FMF in one third of cases. FMF patients can be diagnosed with AA amyloidosis if they are not compliant to colchicine or if daily dose is not sufficient. Preventing amyloidosis is crucial in FMF patients. Colchicine remains the first line treatment to prevent amyloidosis but induced side effect and inobservance. Anakinra seems a good alternative for patient with renal amyloidosis in order to normalize serum concentrations of SAA.
Prognostication of survival and progression to dialysis in AA amyloidosis

G Palladini¹, E Riva¹, ², M Basset¹, F Russo¹, P Milani¹, E Pasquinucci¹, A Foli¹, M. Nuvolone¹, L Obici¹, G Merlini¹

¹Amyloidosis Research and Treatment Center, Foundation IRCCS Policlinico San Matteo, and Department of Molecular Medicine, University of Pavia, Pavia, Italy and ²Hematology Department, Hospital de Clínicas, Facultad de Medicina. Montevideo, Uruguay

giovanni.palladini@unipv.it

INTRODUCTION: In the last decade, availability of accurate diagnostic techniques, recognition of the importance of achieving low SAA concentrations, and accessibility of novel effective treatments for the underlying disease has greatly impacted the management of AA amyloidosis. Here we report the outcome of 200 consecutive patients diagnosed between 1991 and 2015.

MATERIAL & METHODS: Patients were evaluated every 6 months. Treatment was targeted at reduction (if possible normalization) of SAA (available from baseline in 97% of patients). The cutoffs used to dichotomize continuous variables were determined by ROC analyses based on death or dialysis at 4 years.

RESULTS: Median age was 58 years (range 20-83 years) and 40% of patients were males. The underlying disease was arthritis in 31% (rheumatoid arthritis in 20%), inflammatory bowel disease in 13%, infections in 10% (cystic fibrosis 4%, soft tissue infections 4% bronchiectasis 2%), hereditary periodic fever in 8%, Castleman disease in 4%, and other in 2%. In 32% of cases the underlying disease remained unknown. Interestingly, 39% of patients in this latter group were obese, suggesting that obesity-related inflammation might be the cause of AA amyloidosis in a subgroup of patients. The median duration of inflammatory disease before the diagnosis of amyloidosis was 13.7 years. In 5% of patients the identification of the underlying disease followed the diagnosis of amyloidosis. The kidney was involved in 100% of subjects, the heart in 14%, the liver in 8%, and the gastrointestinal tract in 6%. Excluding 10% of patients who were on dialysis at the time of diagnosis, median (range) proteinuria was 3.3 g/24h (0.5-45 g/24h) and eGFR 35 mL/min (4-90 mL/min). With a median follow-up of living patients of 4.4 years, median survival and time to dialysis were 11.9 and 11.2 years, respectively. The variables independently predicting survival were age >55 years (median 6.8 vs. not reached, P<0.001), underlying infection (median 8.9 vs. 11.9 years, P=0.021), and eGFR <45 mL/min (77% vs. 93% at 5 years, P=0.028). Renal survival was independently predicted by proteinuria >4 g/24h (median 6.7 vs. 16.5 years, P=0.004), and eGFR <35 mL/min (median 5.9 vs. 14.3 years, P<0.001). Two staging systems were generated (Figure 1). Baseline SAA did not predict the outcome; however, the ability to reach a low SAA concentration (<10 mg/L) at 6 months independently improved renal survival (median 5.3 vs. 13.7 years, P=0.010). No deaths were observed in the 17 patients treated with biologic agents upfront, but statistical significance was not reached due to low numbers (P=0.059).

DISCUSSION & CONCLUSIONS: Early diagnosis, at a stage when renal function is not irreparably compromised, and effective therapy are the keys to improve the outcome of patients with AA amyloidosis.

![Fig. 1. Staging of AA amyloidosis. A) Overall survival; staging based on age >55 years, underlying infection, and eGFR <45 mL/min. B) Renal survival; staging based on proteinuria >4 g/24h and eGFR <35 mL/min.](image-url)
Transcriptional upregulation and sequence variation in the SAA gene pathway in island foxes (*Urocyon littoralis*) with AA amyloidosis

PM Gaffney¹, T Gaasterland², CJ Sigurdson¹

¹Department of Pathology, School of Medicine, University of California, San Diego, La Jolla, CA, USA. ²Scripps Institution of Oceanography and Institute for Genomic Medicine, University of California, San Diego, La Jolla, CA, USA

pmgaffney@ucdavis.edu

INTRODUCTION: Amyloid A (AA) amyloidosis is highly prevalent (34%) in the endangered island fox (*Urocyon littoralis*), causing morbidity and mortality and threatening species recovery. AA amyloidosis occurs secondary to chronic inflammation and prolonged elevation of the acute phase protein, serum amyloid A (SAA). Island foxes have a high burden of endemic chronic infectious diseases (88%) and an amyloidogenic AA protein sequence, yet not all animals develop AA amyloidosis. Additionally, island foxes have evolved in genetic and geographic isolation for 10,000 years, have low genetic diversity, and there are no reports of amyloidosis in the nearest genetic relative of the island fox, the mainland gray fox. An underlying genetic predisposition to disease is suspected.

MATERIAL & METHODS: The purpose of this study is to utilize the island fox as a natural model of AA amyloidosis and compare SAA serum levels (ELISA), differential gene expression (q-RT-PCR) and sequence variation in foxes with and without AA amyloidosis. For genetic sequence analysis, we utilize high-throughput gene sequencing (RNA-seq, Exome-seq) and novel bioinformatic approaches developed de-novo for this non-model species.

RESULTS: Of 70 necropsy cases, hepatic SAA and C/EBP-δ, and splenic IL-6, IL-1α, IL-1β transcripts were significantly elevated in island foxes with AA amyloidosis (p < 0.05), suggesting increased SAA transcription by hepatocytes through an IL-6 mediated pathway. Higher SAA transcripts also correlated with more severe disease (p < 0.02). Serum SAA protein concentrations were similar between foxes with and without amyloidosis; therefore, serum protein concentration may not reflect disease status. Using the *Canis familiaris* genome, CanFam3, and one annotated SAA gene, we defined the complete SAA gene family of dog to compare to island and gray fox. Of five identified SAA genes, four are structurally homologous to SAA1, SAA2, SAA3 and SAA4 of humans and mice (Figure 1). From genomic DNA, the fox SAA gene family contains 776 SNPs, five of which are in the promoter region of SAA2 within the binding site sequences for C/EBP and NF-κB. Remarkably, the 6 island fox subspecies and gray fox could be differentiated phylogenetically based on the SAA gene family SNPs. Capture and sequencing of island and gray fox exomes using canine-based probes revealed 9364 SNPs unique to island foxes with AA amyloidosis compared to gray foxes. RNA-seq analysis revealed differential gene expression in over 100 genes when comparing foxes with and without disease.

DISCUSSION & CONCLUSIONS: The genetically isolated island fox has a high prevalence of AA amyloidosis associated with an upregulation of pro-inflammatory cytokines and genes in the SAA transcription pathway. Genetic variation in the fox SAA gene promoter supports examination of a functional role of fox-specific SNPs. Differential gene expression between foxes with and without disease may lead to the discovery of pathways associated with disease and a novel mechanism for AA amyloidosis.

![Image of SAA gene family](https://via.placeholder.com/150)

**Fig.1:** Island and gray foxes have 776 SNPs in the SAA gene family region relative to canine. Five SNPs are present in the promoter region of SAA2 within the binding site sequences for C/EBP and NF-κB.
A clinical Phase 3 confirmatory trial of Kiacta™ in the treatment of AA amyloidosis

G Merlini1, D Garceau2, L M Dember3, T Sablinski2, H Lachmann4, JL Berk5, L Obici1 on behalf of the Kiacta study investigators

1 University Hospital San Matteo, Pavia, Italy, 2 Auven Therapeutics, New York, USA, 3 University of Pennsylvania, Philadelphia, USA, 4 Royal Free and University College Medical School, London, United Kingdom, 5 Boston University, USA

INTRODUCTION:

Kiacta (eprodisate) is a member of a new class of agents that inhibits the development of tissue amyloid deposits in mouse models of AA amyloidosis1. In a first clinical Phase 2/3 study conducted in 183 AA patients, Kiacta administered for 2 years reduced the risk of renal function deterioration and death by 42% as compared to placebo (Cox proportional hazards regression analysis: HR=0.58; p=0.025)2. In this study, Kiacta was shown to be safe and well tolerated. To confirm the safety and efficacy of Kiacta in the treatment of AA amyloidosis, a clinical Phase 3 trial has been conducted in 261 AA patients from 2010 to 2016.

MATERIAL & METHODS:

The clinical Phase 3 confirmatory trial was an international, multicentre, randomized, double-blind and placebo-controlled study. Patients with AA amyloidosis and kidney involvement were randomized to Kiacta or placebo in a 1:1 ratio. The diagnosis of AA amyloidosis required histologic confirmation (Congo red staining and immunohistochemistry/immuno-electron microscopy). Presence of renal involvement was defined by proteinuria ≥ 1 g/day. Patients with kidney diseases other than AA amyloidosis and creatinine clearance ≤ 25 ml/min/1.73m² were excluded from the study. This was an event-driven trial targeting 120 patient-events of renal deterioration as defined by either a persistent ≥ 80% increase in serum creatinine (SCr), a persistent ≥ 40% decrease in creatinine clearance (CrCl) or progression to end stage renal disease (ESRD). The primary efficacy analysis will compare the time to first event of renal deterioration using the Log rank test. Patient’s renal function was assessed every 3 months throughout the duration of the study.

RESULTS:

Between May 2011 and January 2015, 462 AA patients were screened and 261 were enrolled in 61 study sites from 30 countries. The most frequent reasons for screening failure were CrCl ≤ 25 ml/min/1.73m² (35%), negative biopsy (24%) and proteinuria < 1 g/day (22%). The greater number of patients have been recruited, in decreasing order, from East Europe, Middle East/Asia, West Europe and Americas. A total of 73 patients discontinued the study prematurely, 28 due to death. 31 patients progressed to ESRD during the study. The median time of follow-up during the study was 24 months with a maximum of 57 months. An independent Data Safety Monitoring Board (DSMB) has reviewed patient safety throughout the duration of the study and never raised any safety concerns. The target of 120 patient-events was reached in January 2016, ending study activities. We anticipate the top-line study results to be available in Q2 2016 after the resolution of queries and database cleaning. Results of this Phase 3 study will be presented at the XV ISA Symposium.

DISCUSSION & CONCLUSIONS:

This phase 3 study along with the first Phase 2/3 trial constitute the largest prospective and well-controlled studies ever conducted in systemic amyloidoses. It will also provide the most comprehensive data on the natural course of AA amyloidosis. If results are positive, this Phase 3 confirmatory trial may provide the basis for the approval of the first treatment for AA amyloidosis.

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PASSIVE TREATMENT WITH MONOCLONAL ANTIBODIES TO ISLET AMYLOID POLYPEPTIDE SIGNIFICANTLY IMPROVED BOTH HEMOGLOBIN A1C AND GLUCOSE TOLERANCE AND DECREASED EXTRACELLULAR AMYLOID DEPOSITS IN THE TRANSGENIC HIP RAT MODEL OF TYPE 2 DIABETES


Prothena Biosciences Inc, South San Francisco, California, USA.

wagner.zago@prothena.com

INTRODUCTION: Type 2 diabetes (T2D) is the most common form of diabetes. It affects 150 to 250 million persons worldwide, a population that is forecast to double by 2025. The disease increases patients’ risks for heart attack, stroke, kidney failure, and blindness and may shorten lifespans by up to 15 years. No available class of antidiabetes medications changes the course of the disease. We sought to assess an immunotherapeutic approach to T2D by targeting islet amyloid polypeptide (IAPP), a 37-amino acid peptide that is co-secreted with insulin and is highly prone to aggregation. IAPP amyloid deposits are found in most T2D pancreata, and the toxicity of these insoluble and soluble aggregates likely contributes to the pathophysiology of T2D. We evaluated the effects of anti-IAPP antibodies in a transgenic rat that expresses human IAPP (HIP rats). HIP rats present T2D-relevant phenotypes such as loss of pancreatic β cells and extracellular IAPP deposition.

MATERIAL & METHODS: We developed monoclonal antibodies targeting different epitopes on IAPP and assessed the effects of passive immunotherapy on a variety of end points. Antibody specificity and affinity to misfolded IAPP was characterized by BIACore analysis. HIP rats (10-12 weeks old) were injected intraperitoneally once a week with 10 mg/kg anti-IAPP or isotype control antibodies. Blood samples were assessed for hemoglobin A1C (HbA1C) and oral glucose tolerance during the live phase. After 30 weeks of treatment animals were sacrificed, and the pancreata were removed and processed for histology.

RESULTS: Passive immunotherapy with a subset of anti-IAPP antibodies promoted significant improvement in HbA1C levels and the area under the curve in the oral glucose tolerance test compared with passive immunotherapy with an isotype control antibody. Furthermore, evidence of decreased extracellular IAPP amyloid deposits and preservation of β cells was observed with a subset of antibodies.

DISCUSSION & CONCLUSIONS: This study demonstrates that passive anti-IAPP immunotherapy with antibodies that target specific epitopes of the misfolded protein suppresses the development of a T2D-like phenotype in a rat model. Anti-IAPP immunotherapy may thus hold therapeutic potential as a disease-modifying approach in T2D.
O50
Prevalence of monoclonal gammopathy of unknown significance in ATTR-wild type patients
HI Geller, A Singh, TM Mirto, DK Dupee, R Padera, JP Laubach, RH Falk

Brigham and Women’s Hospital Cardiac Amyloidosis Program, Boston MA, USA
Brigham and Women’s Hospital Cardiac Amyloidosis, Boston MA, USA
Dana Farber Cancer Institute, Boston MA, USA
Higeller@partners.org

INTRODUCTION: The prevalence of monoclonal gammopathy of unknown significance (MGUS) in the general population increases with increasing age; 3.2% at 50 yrs and older, 5.3% at 70 yrs and older and 7.5% at 85 yrs and older (1). Wild type TTR amyloidosis (ATTRwt) is a disease of elderly men typically 70 years or older and thus MGUS would be expected to co-exist with ATTRwt in 5-8% of cases. Several authors have noted a higher than expected prevalence of MGUS in the ATTRwt population (2, 3), but this finding has not been precisely defined. We therefore sought to determine the prevalence of MGUS and abnormal free light chain ratio (κ/λ ratio) in a series of patients with ATTRwt cardiac amyloidosis. As patients with AL amyloidosis have an easily identifiable monoclonal gammopathy in about 90% of cases, usually with an abnormal κ/λ ratio, the finding of MGUS in ATTRwt may be confusing for the uninitiated general physician.

MATERIAL & METHODS: 123 patients (average age 75.6 +/- 7.8 yr, 4 female) with proven ATTRwt cardiomyopathy were seen over a 10 year period. All had a record of serum free light chains (SPEP) and serum immunofixation (IF). 102 (83%) were diagnosed with ATTRwt on the basis of a biopsy with immunohistochemistry using antibodies to TTR, kappa and lambda. In equivocal and uncertain cases (n=26) amyloid type was determined by mass-spectrometry. 17 were diagnosed on the basis of PYP scanning and absent MGUS with clinically isolated cardiac amyloidosis. Normal values for serum kappa were 3.3-19.4 mg/L and for serum lambda 5.7-26.3 mg/L with a ratio of 0.26-1.65.

RESULTS: 101 (82%) patients showed no evidence of MGUS on IFE and/or SPEP, 4 of whom had hypergammaglobulinemia and 1 hypogammaglobulinemia. Of the 22 (18%) patients with MGUS, 9 (40.9%) had an abnormal κ/λ ratio (8 with kappa and 1 with lambda predominance, 1 of whom (free kappa = 169 mg/dL) met criteria for smoldering myeloma). Of the population without MGUS on IF/SPEP, 16 (15.8%) presented with abnormal κ/λ ratio (15 with kappa and 1 with lambda predominance). As renal impairment may disproportionately affect kappa levels, we compared serum creatinine levels in those with and without an abnormal κ/λ ratio. No significant difference was found between mean creatinine of those with abnormal κ/λ ratio and MGUS (group 1) and those with abnormal κ/λ ratio and no MGUS (group 3) or between those with normal κ/λ ratio and MGUS (group 2) and those with normal κ/λ ratio and no MGUS (group 4) (P= 0.57, P=0.73).

DISCUSSION & CONCLUSIONS: MGUS was present in 18% of patients with ATTRwt cardiomyopathy, greater than 40% of whom also have an abnormal κ/λ ratio. Unlike AL amyloidosis, the abnormal κ/λ ratio in ATTRwt when present is almost exclusively kappa predominant as expected in a general MGUS population. Renal impairment did not appear to account for elevated free kappa in this population even in the absence of MGUS.

REFERENCES:

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Table 1. Mean serum creatinine in all groups
Amyloidosis derived from apolipoprotein C-II: a novel type of renal amyloidosis

SH Nasr¹, S Dasari¹, L Hasadsri¹, JD Theis¹, JA Vrana¹, MA Gertz¹, P Muppa¹, MT Zimmermann¹, KL Grogg¹, A Dispenzieri¹, S Sethi¹, WE Highsmith¹, G Merlini², N Leung², PJ Kurtin¹

¹Mayo Clinic, Rochester, MN, USA. ²University of Pavia, Pavia, Italy.
nasr.samih@mayo.edu

INTRODUCTION: Most renal amyloidosis cases are AL, AA or ALect2, but several rare hereditary forms can involve kidneys; about 2% are currently unclassified.

MATERIAL & METHODS: We recently encountered a 61-year-old female who presented with nephrotic syndrome and renal impairment. Renal biopsy revealed amyloidosis with predominant involvement of glomeruli and medullary interstitium.

RESULTS: Proteomic analysis of Congo red positive deposits detected large amounts of APOC2 protein. DNA sequencing of APOC2 gene in the patient and one of her children detected a heterozygous c.206A→T transition, causing a E69V missense mutation. We also detected the mutant peptide in the proband’s renal amyloid deposits. We performed generalized Born implicit solvent molecular dynamics simulations to assess the effect of the mutation on APOC2 protein folding. The mutation is located in the linker region and it alters the native confirmation of the protein which could destabilize the protein and lead to amyloid formation.

We subsequently identified, by proteomics, 7 additional elderly patients with APOC2 rich amyloid deposits, all of whom had kidney involvement. To rule out the possibility that APOC2 can be co-deposited in renal amyloid deposits of other types or be present in glomeruli of non-amyloid glomerular diseases, we utilized normalized MS/MS spectral counts to estimate the relative abundance of APOC2 protein in the glomerular amyloid deposits of all AApoCII cases (n=8), Congo red-positive deposits in kidneys of other amyloid subtypes (n=532), glomeruli of patients with non-amyloid glomerular disease (n=49) and normal glomeruli of healthy individuals (n=10). We detected APOC2 protein only in amyloidosis cases of AApoCII type. Immunohistochemical staining for APOC2 was strongly positive in all 3 AApoCII cases tested and was negative in 7 cases of renal amyloidosis of other types (AL-1 n=2, AL-k n=1, AA n=1, AApoAIV n=3).

Our total cohort of AApoCII renal amyloidosis consisted of 5 females and 3 males, all elderly at diagnosis (median age 70 years). Patients presented with proteinuria (median 24h urine protein 3.4 g/day) including 43% with full nephrotic syndrome, with or without renal insufficiency (median serum creatinine 1.8 mg/dl). Histologically, glomeruli were affected in all cases. While the index case showed global glomerular involvement, the remaining 7 cases exhibited a distinctive nodular glomerular involvement (Fig 1). Cortical and medullary interstitial involvement was present in 25% and 40% of cases, respectively. Electron microscopy showed the typical ultrastructural appearance of amyloid. Immunofluorescence was negative for immunoglobulin light and heavy chains.

DISCUSSION & CONCLUSIONS: While prior in vitro studies showed that APOC2 can form amyloid fibrils and that certain mutations in the protein can promote amyloid fibrillogenesis, there have been no reports of this type of amyloidosis occurring in humans. We report a new form of human hereditary amyloidosis (AApoCII) that is derived from APOC2 protein and appears to manifest in the elderly and preferentially affect the kidneys.

Fig. 1. Renal biopsies from 2 patients with AApoCII demonstrating nodular glomerular involvement by amyloid (H&E)
Renal amyloidosis associated with a novel apoC-II variant

L Obici¹, G Palladini¹, S Casarini¹, PJ Kurtin², JD Theis², S Dasari³, L Verga¹, V Pirruccello¹, GL Capello³, M Nuvolone¹, G Merlini¹

¹Amyloidosis Research and Treatment Center, Foundation IRCCS Policlinico San Matteo, and Department of Molecular Medicine, University of Pavia, Pavia, Italy. ²Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, USA. ³Department of Pathology, University of Pavia, Pavia, Italy

INTRODUCTION: Several human apolipoproteins are known to be amyloidogenic in vivo, either in their native form or as a consequence of destabilizing mutations, the latter resulting in hereditary systemic amyloid diseases. We describe a novel form of renal amyloidosis associated with a rare apolipoprotein C-II mutation identified in eight patients from five unrelated Italian families.

MATERIAL & METHODS: Amyloid typing was performed by proteomic analysis and immunoelectron microscopy on kidney biopsies available from four patients. Genetic analysis was performed by Sanger sequencing of the four exons and exon-intron boundaries of the APOC2 gene (NCBI RefSeq accession number NM_000483.4).

RESULTS: All patients presented with nephrotic syndrome with or without renal insufficiency since the sixth decade of life. Family history was present in three index cases. No signs of cardiac, liver or neurological involvement were found. Kidney biopsies disclosed large glomerular amyloid deposits in all patients, with no significant vascular and medullary involvement.

LMD-LC-MS/MS performed on CR positive deposits detected apolipoprotein C-II and universal amyloid tissue biomarkers (APOE, APOA4, and SAP). Immunelectron microscopy confirmed specific immunostaining of amyloid fibrils with a polyclonal anti-apoC-II antibody.

DNA analysis identified a single-base change (c.122C>A) in exon 3 of APOC2, resulting in a Threonine for Lysine substitution at residue 19 of the mature protein. The mutation was identified in 1/200 control chromosomes. Lipid profile showed occurrence of hypercholesterolemia without hypertriglyceridemia, consistent with nephrotic syndrome.

DISCUSSION & CONCLUSIONS: Native apoC-II accumulates in amyloid deposits of atherosclerotic lesions and its amyloidogenic properties have been largely characterized in vitro. Lipids substantially affect misfolding and aggregation of apoC-II, consistent with the physiological lipid binding function of the protein [1]. Here we show that apoC-II becomes responsible for a systemic form of amyloid disease in the presence of an amino acid replacement located in the lipid binding domain of the protein. This mutation has been previously reported as a rare variant (MAF <1% in controls) in hyperlipidemic patients, but no clear association with specific lipid abnormalities has been established to date [2]. The unexpected identification of this variant in five families from different geographic areas, together with its established occurrence at variable frequency in different populations suggests that the prevalence of this form of amyloidosis might be higher than anticipated. Moreover, the apparently incomplete penetrance of this mutation supports the role of other genetic, epigenetic or environmental factors accounting for variable disease expressivity, possibly affecting protein interactions with lipids along its metabolic pathway.

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Liver transplantation in treatment of TTR amyloidosis

Bo-Göran Ericzon

Karolinska Institutet, Stockholm, Sweden

ABSTRACT

Introduction: Liver transplantation (LTx), introduced in 1990, has served for more than two decades as the only available treatment with capacity to halt the progress of disease in transthyretin amyloidosis. For the most common variant, Val30Met, the effect of liver replacement is well known leading to stabilization in the majority of patients. However, not all patients are helped by transplantation. Progress of cardiac amyloidosis is not uncommon necessitating both liver and heart transplantation (LTX/HTx) for some patients. The effect of LTx is less well studied in patients with non-Val30Met mutations, and outcome has generally been inferior to that seen in patients with the Val30Met mutation. Large variations in survival, not only between different mutations but also between mutations with similar phenotypes, have been noted and it is clear that each mutation needs to be considered individually. Some mutations have similar long-term survival as the Val30Met, while LTx is not to be recommended for other mutations. Several novel pharmaco-therapeutical approaches have emerged over the last years and may provide a more attractive and less invasive treatment for this patient population.

Methods: Data concerning outcome after LTx for ATTR amyloidosis was extracted from the FAPWTR registry. Survival rates were analyzed by the Kaplan-Meier method and Log-Rank test.

Results: In total, 58 different mutations were treated by LTx alone or by LTX/HTx. Data from more than 2000 patients were accumulated from 77 collaborating liver transplant centers. Overall, 20-year survival after LTx was 55.3%. Modified Body Mass Index, early onset of the disease, disease duration before transplantation and Val30Met versus non Val30Met mutations were independent significant survival factors. Cardiovascular death was markedly more common than that observed in patients undergoing LTx for end stage liver disease. There has been a significant drop in the annual number of transplants over the last years following the introduction of novel pharmacotherapy directed towards TTR amyloidosis in Europe. A careful evaluation regarding the effect of new promising pharmacotherapies in relation to LTx is of the outmost importance. There is a risk, that patients not responding to pharmacotherapy may be exposed to a less favorable surgical outcome because LTx is delayed. Furthermore, it is unknown if patients not responding to LTx will respond better to alternative treatment and vice versa.

Conclusion: Long-term survival after LTx for many TTR variants is excellent. Several new promising pharmacological treatments are under evaluation. In order to determine the most optimal use, these new approaches must be compared to the existing surgical therapy. Perhaps the best treatment for some patients will be a combination of pharmacotherapy and surgery.

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Cardiac Transplantation for Amyloidosis

MS Maurer

Division of Cardiology, Center for Advanced Cardiac Care, Columbia University Medical Center, New York Presbyterian Hospital

msm10@cumc.columbia.edu

Cardiac involvement in systemic amyloidosis is associated with a poor prognosis especially in advanced stages of disease. Despite the progress in our understanding of the basic biologic mechanisms that have led to an emergence of new therapies for both AL and TTR amyloidosis, the prognosis of patients with advanced cardiac amyloidosis remains poor and has not changed over the past few decades. Accordingly, there has been an increase in the number of patients undergoing cardiac transplantation for both AL and TTR amyloidosis. Initial experiences with cardiac transplantation for AL amyloid were hindered by recurrent amyloid in the graft and poor outcomes. However, with the advent of a growing armamentarium of plasma cell therapies and the use of stem cell transplantation with high dose chemotherapy, contemporary series suggest that outcomes of patients with AL amyloid treated with cardiac transplantation do not differ from those transplanted for similar non-amyloid indications. In this review, we will highlight the rationale for pursuing cardiac transplantation in selected candidates with cardiac amyloidosis, the obstacles given the current allocation schemes faced by patients who are waiting for cardiac transplantation including the high wait list mortality of patients with AL amyloid and their need for priority listing given the inability to be bridge with current mechanical support. Discussion will include guidelines based recommendations from transplant societies regarding the eligibility of patients with cardiac amyloid for cardiac transplantation, the selection process for evaluating and listing such patients and their outcomes post-transplant with a focus on the comparison between AL and TTR cardiac amyloid. Emerging data from multicentre cohort studies including the International Consortium for Cardiac Amyloid Transplantation (iCCAT) which includes ten centers in the United States and Europe (Massachusetts General Hospital, Columbia University Medical Center, Houston Methodist Hospital, Stanford University Medical Center, University of Padova, Newark-Beth Israel Medical Center, Boston University Medical Center, Cedars-Sinai Medical Center, Cleveland Clinic and University of California at San Francisco) that have pooled their data will be presented. Encouragingly data suggests that the outcomes for patients with AL amyloid undergoing cardiac transplantation followed by either high dose chemotherapy/stem cell transplant or targeted plasma cell therapy are improving over time. Additionally, data suggests that outcomes for TTR cardiac amyloid subjects who receive a cardiac transplant are better than for AL cardiac amyloid subjects undergoing cardiac transplant. Ongoing areas for future research to address unanswered questions and policy changes in allocation of organs will be delineated. The real ultimate goal is to facilitate early identification of patients with AL cardiac amyloid and TTR cardiac amyloid and employ targeted biologically active therapies in preventing cardiac dysfunction in order to eliminate the need for orthotropic heart transplantation.
Transthyretin-type cerebral amyloid angiopathy in post-transplant patients with hereditary ATTR amyloidosis: Correlates between clinical findings and PIB-PET imaging

Y Sekijima, M Yazaki, K Oguchi, N Ezawa, T Yoshinaga, S-I Ikeda

Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan. Institute for Biomedical Sciences, Shinshu University, Matsumoto, Japan. Jisenkai Brain Imaging Research Center, Matsumoto, Japan. sekijima@shinshu-u.ac.jp

INTRODUCTION
Liver transplantation markedly improves survival in hereditary ATTR amyloidosis [1,2]. However, the prolonged disease duration induces de novo central nervous system (CNS) amyloidosis, ATTR-type cerebral amyloid angiopathy (CAA), as choroid plexus continues to produce variant transthyretin [3,4]. We investigated the prevalence and clinical features of post-transplant CNS symptoms in hereditary ATTR amyloidosis patients and their Pittsburgh compound B (PIB)-positron emission tomography (PET) imaging correlates.

MATERIAL & METHODS
We monitored prevalence and type of CNS symptoms in 53 consecutive post-transplant patients with hereditary ATTR amyloidosis. 11C-PIB-PET was performed in 15 patients with various disease durations. We also analyzed pathological and biochemical characteristics of ATTR amyloid deposition in the brain of a post-transplant patient.

RESULTS
Transient focal neurological episodes (TFNEs) attributed to ATTR-type CAA were found in 11.3% of post-transplanted hereditary ATTR amyloidosis patients. TFNE occurred on average 16.8 years after onset of the disease. Patients with longer duration of illness (≥ 10 years) showed increased 11C-PIB retention in the brain. The 11C-PIB accumulation pattern in hereditary ATTR amyloidosis was unique and completely different from those in Alzheimer’s disease or Aβ-type CAA. In the autopsy case, ATTR amyloid deposition was mainly localized to leptomeningeal vessels and leptomeninges of the brain. Amyloid fibrils in the brain were almost completely composed of variant TTR.

DISCUSSION & CONCLUSIONS
TFNE due to ATTR-type CAA occurred frequently in post-transplant patients with long disease durations. 11C-PIB-PET is a useful diagnostic tool for ATTR-type CAA. ATTR amyloid deposition in the CNS, as measured by PIB-PET, was detected approximately 10 years before onset of TFNE.

REFERENCES:
Evaluation of central nervous complications following liver transplantation in patients with hereditary transthyretin amyloidosis

Wange N1, Pilebro B1, Wixner J1, Ericzon B-G2, Suhr OB1

1Department of Public Health and Clinical Medicine, Umeå University, Umeå, and 2Division of Transplantation surgery, Karolinska University Hospital, Huddinge, Sweden

INTRODUCTION

Hereditary transthyretin amyloid (h-ATTR) amyloidosis is a fatal systemic amyloidosis caused by a mutation in the transthyretin (TTR) gene. Liver transplantation (LTx) is the only available treatment proven to increase survival in these patients. A recent study noted a high risk for central nervous (CNS) complications among transplanted h-ATTR amyloidosis patients (1). The suggested cause was local mutant TTR production in the brain’s choroid plexus leading to cerebral amyloid angiopathy (CAA). We aimed to investigate the incidence of CNS-complications among liver transplanted h-ATTR amyloidosis patients in Sweden and their relationship to heart arrhythmia, sex, ischaemic heart disease, disease duration, cardiomyopathy and age at onset of disease.

MATERIAL & METHODS

Liver transplanted h-ATTR amyloidosis patients in Västerbotten county surviving more than 3 years after LTx were included (N=63). Data was collected through medical records and radiological surveys. CNS-complications were defined as ischaemic stroke, transient ischaemic attack (TIA), intra-cerebral haemorrhage (ICH), subarachnoid haemorrhage (SAH), epileptic seizure, dementia and migraine. Only CNS-complications that debuted after LTx were included. Classification was according to the diagnosis obtained at the local hospital during follow-up.

RESULTS

Forty % (25/63) of the patients suffered from one or more CNS-complication which increased with disease duration. Twenty-seven % (17/63) had cerebrovascular (CVD) events, defined as ischaemic stroke, TIA, ICH or SAH. Thirty-three % (21/63) of the patients had atrial fibrillation (AF) which was strongly associated to CVD events (p = 0.002). A significantly higher occurrence of AF was seen in the group with late onset of h-ATTR amyloidosis versus early onset (p = 0.00006). The only significant regressor in the multivariate analysis for CVD-events was AF (Table 1).

DISCUSSION AND CONCLUSIONS

CNS-complications are commonly observed in liver transplanted h-ATTR amyloidosis patients, and increase steadily with time after transplantation. A majority of these are of cerebrovascular origin. The prevalence of AF is high in LTx patients with CVD and, thus, embolisation rather than amyloid angiopathy may be the main cause. A more intense screening for AF and subsequent anticoagulation therapy to prevent cerebral embolic events needs to be considered.

Table 1. Univariate and Multivariate analysis of factors with an impact on cerebrovascular complications after liver transplantation in hereditary V30M ATTR amyloidosis patients

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<td>Sex (male/female)</td>
<td>1.1 (0.5 – 2.3)</td>
<td>0.853</td>
<td>2.0 (0.6 – 6.5)</td>
<td>0.240</td>
</tr>
<tr>
<td>Late onset1</td>
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<td>0.302</td>
<td>0.6 (0.1 – 2.9)</td>
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</tr>
<tr>
<td>Cardiomyopathy2</td>
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<td>0.041</td>
<td>2.2 (0.7 – 7.3)</td>
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<td>Hypertension</td>
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<td>Atrial fibrillation</td>
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<td>4.3 (1.3 – 14.2)</td>
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<tr>
<td>Ischaemic heart disease</td>
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<td>0.019</td>
<td>6.1 (0.8 – 45.5)</td>
<td>0.080</td>
</tr>
</tbody>
</table>

1Disease onset at age >50 years. 2 Echocardiographic end-diastolic septum thickness >12 mm

Polyneuropathy after FAP domino liver transplantation

G Solders¹, G Nowak², M Wiss², B-G Ericzon²

¹ Department of Clinical Neurophysiology, Karolinska University Hospital, Karolinska Institute, Stockholm, Sweden. ² Department of Transplantation Surgery, Karolinska University Hospital, Karolinska Institute, Stockholm, Sweden.

goran.solders@karolinska.se

INTRODUCTION: Domino liver transplantation (DLT) using grafts from patients with familial amyloidotic polyneuropathy (FAP) is an established procedure. Knowledge of the long-term outcome of DLT including the risk of developing de novo polyneuropathy as a consequence of the DLT procedure is important so that potential DLT recipients can be correctly informed about the risks involved with the procedure.

MATERIAL & METHODS: Prospective postoperative neurological monitoring of peripheral nerve function was performed 1, 5 and 10 years after the transplantation using electroneurography (ENeG). An ENeG index (mean SD correlated to age and/or height) based on 12 parameters was calculated. Also, to assess thin fibre function thermal perception thresholds were measured.

RESULTS: 63 DLTs using FAP grafts were conducted between 1997 and 2015. Three patients underwent DLT twice. The 5-year patient survival in hepatocellular carcinoma (HCC) patients (n = 23) and non-HCC patients (n = 27) was 30 % and 52 % respectively. Thirteen of 29 deaths were related to tumor recurrence. De novo signs of polyneuropathy was found in the ENeG recording in 3/27 (11 %) patients after 5 years and in 3/7 (43 %) patients after 10 years. The mean EneG index deteriorated from -1,04 (normal ±0,72) at year 1, to -1,53 at year 5, and -1,60 at year 10. Parameters reflecting axonal function and C-fibre function were the most affected.

DISCUSSION & CONCLUSIONS: Survival after DLT was good except in patients with advanced HCC. In a proportion of DLT recipients, the development of impaired nerve conduction may indicate the emergence of de novo amyloidosis polyneuropathy. This appears to occur earlier in recipients of FAP livers than in the natural course of native FAP.

REFERENCES:

Cardiac dysautonomia predicts long term survival in hereditary transthyretin amyloidosis after liver transplantation

Vincent Algalarrondo1, 7 Teresa Antonini2, 7, Marie Théaudin3, 7, Denis Chemla4, Anouar Benmalek5, Catherine Lacroix5, Denis Castaing2, 7, Cécile Cauquil3, 7, Sylvie Dinanian1, 7, Ludwine Eliahou1, 7, Didier Samuel2, 7, David Adams3, 7, Dominique Le Guludec6, Michel S Slama1, 7, François Rouzet6

prmslama@gmail.com

1: Cardiology department, Antoine Béclère hospital, Assistance Publique Hôpitaux de Paris (AP-HP), UMR-S 1180, Univ. Paris-Sud, Clamart, France. 2: Hepato-Biliary Center, Paul Brousse hospital, AP-HP, UMR-S 785, Univ. Paris-Sud, Villejuif, France. 3: FILNEMUS, Neurology Department, Kremlin Bicêtre hospital, AP-HP, Bicêtre, France. 4: Physiology Department, EA4533, Univ. Paris-Sud, Le Kremlin Bicêtre, France 5: School of Pharmacy, Univ. Paris-Sud, Chatenay Malabry, France 6: Nuclear medicine Department and DHU FIRE, Bichat Claude Bernard hospital, AP-HP, Univ Paris VII, U1148, Paris, France.7: French Referral Center for FAP and Other Rare Peripheral Neuropathies (NNERF)

Objectives: To compare techniques evaluating cardiac dysautonomia in predicting the risk of death of patients with hereditary transthyretin amyloidosis (mATTR) after liver transplantation (LT).

Background: mATTR is a multisystemic disease involving mainly the heart and the peripheral nervous system. LT is the reference treatment and preoperative detection of high risk patients is critical. Cardiovascular dysautonomia is commonly encountered in ATTR and may affect patients’ outcome, although it is not known yet which technique should be used in this field.

Methods: In a series of 215 consecutive mATTR patients who underwent LT, cardiac dysautonomia was assessed by a dedicated clinical score, time-domain heart rate variability, 123-MIBG H/M ratio on scintigraphy and heart rate response to atropine (HRRA).

Results: Patient’s median age was 43 years, 62% were men and 69% carried the Val30Met mutation. Cardiac dysautonomia was documented by at least one technique for all patients but 6 (97%). In univariate analysis, clinical score, 123-MIBG H/M ratio and HRRA were associated with mortality but not heart rate variability. 123-MIBG H/M ratio and HRRA had greater AUC of ROC curves than clinical score and heart rate variability (respectively, AUC: 0.787; 0.748; 0.656 and 0.523). Multivariate score models were then built using the following variables: NYHA class, interventricular septum thickness and either 123-MIBG H/M ratio (S_MIBG) or HRRA (S_atropine). AUC of S_MIBG and S_atropine were greater than AUC of univariate models, although non significantly (respective AUC: 0.798 and 0.799). Predictive powers of S_MIBG and S_atropine and a reference clinical model (AUC: 0.785) were similar.

Conclusions: Evaluation of cardiac dysautonomia is crucial to predict survival of mATTR patients following LT. Amongst the different techniques that evaluate cardiac dysautonomia, 123-MIBG scintigraphy and heart rate response to atropine had better prognostic accuracy. Multivariate models did not improve significantly prediction of outcome.
Animal models of the human amyloidoses revisited

Joel N. Buxbaum

1Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA, USA

jbux@scripps.edu

Animal models of the human amyloidoses were originally developed in attempts to understand the pathophysiology of the systemic disease(s). It was believed that once that goal was achieved, ultimately Koch’s postulates regarding disease etiology would be fulfilled and such models could be used to define and test potential therapies prior to human trials. The last of these objectives has been reached in the last decade with both biologic and chemically based therapies being explored in various animal models of the systemic amyloidoses AL, ATTR, AA and to a lesser extent Agel, although in some instances the animal model phase of testing was bypassed in favor of human clinical trials.

Despite their utility in testing various therapies, animal models have been less yielding with respect to understanding the mode of tissue damage. While the older view that organ compromise was related to tissue displacement by either fibrillar or non-fibrillar deposits with little inflammatory response, cell culture studies of cytotoxicity, initially in the case of Aβ, then extended to TTR and other precursors, have suggested that the toxic effects of oligomers may be more significant, either alone or in combination with the mechanical effects of the deposits. Recently the process of toxicity has been extended to other model organisms such as drosophila, nematodes and zebrafish. While these have the advantage of an organismal setting it remains to be seen whether they add power to that of the cultured cell assays that have been used in both high throughput screening for possible therapeautic agents and the study of mechanisms of tissue damage.

In 1999 in a review of animal models of the human amyloidoses I pointed out that in order to fully understand disease pathogenesis there were some phenomena that had to be studied at the level of the intact organism, be it intact humans or relevant animal systems. That statement still applies.

Questions which remain open include:

1. Why doesn’t an amyloidogenic protein, present from birth (at least) in similar concentrations, form fibrillar deposits until late in life?

2. Why do deposits occur in some tissues and not in others?

3. What are the organismal responses to the presence of an amyloidogenic protein?

4. Are such responses systemic or local?

5. Are the host responses salutary or do they amplify the manifestations of disease?

6. In the systemic amyloidoses, in which deposition occurs at a distance from the site of precursor synthesis, how does the precursor get from point A to point B?

7. Can these disorders be transmitted from animal to animal and can there be analogous transmission in man?

With respect to specific amyloidoses since we last visited this subject the induced inflammatory murine model of AA continues to provide information particularly with respect to mouse to mouse transmission. The naturally occurring Sam 8 mouse model of ApoAII deposition also appears to be subject to fecal-oral transmission and it has now become clear that aggregates of Aβ behave in the same way as AA aggregates (formerly known as AEF (amyloid enhancing factor).

In other systemic amyloidoses mice transgenic for human systemic amyloid precursors have emerged as the most tractable experimental models. While these have been most widely exploited in the context of neurodegenerative disorders, particular Alzheimer’s disease, most recently they have proven to be useful in the study of ATTR, AL and most recently Agel. The strengths and weaknesses of the animal models now appear to be much clearer than they were when originally introduced, but they are useful.
Reproducing light chain proteotoxicity in animal models: a window on AL cardiomyopathy

Francesca Lavatelli

Amyloidosis Research and Treatment Center, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

AL amyloidosis is a polymorphic disease in terms of clinical presentation and properties of the causative light chains (LCs). Crucial questions for developing targeted therapies concern the bases of organ targeting and dysfunction in vivo. Investigating these features in a proper experimental environment, with clinically annotated LCs, is crucial to obtain biologically meaningful insights on the bases of proteotoxicity and amyloidogenesis. Given the radical impact of cardiomyopathy in AL amyloidosis, much effort has been focused on the definition of the damage mechanisms in this organ. Recent years have witnessed the development of animal models to investigate light chains-related cardiac dysfunction. These organisms, which include in particular the vertebrate zebrafish (1, 2) and the nematode C. elegans (3), recapitulate a specific aspect of the disease: the proteotoxicity related to the soluble forms of amyloidogenic LCs. Under controlled experimental settings, both animals display functional and anatomical changes in the cardiovascular system (zebrafish) and in the muscular cells of the pharynx (C. elegans; this organ in the worm is considered an ortholog of the vertebrate heart) upon exposure to exogenous cardiotoxic LCs. Although these organisms are taxonomically distant from mammalians, they are both widely accepted models in the investigation of protein misfolding diseases; their validity to study AL cardiomyopathy is indicated by the fact that the capacity of the various LCs to cause damage in these animals parallels their ability to be cardiotoxic in humans. In particular, only the LCs from AL cardiomyopathy patients elicit alterations, consisting in reduction of the pharyngeal pumping rate and life span in the worm, and in impaired cardiac function, increased cell death and early mortality in the fish. This damage, in which mitochondrial dysfunction seems to play a major role, occurs without evidence of amyloid fibril deposition, and is not observed using non-cardiotoxic LCs.

This evidence indicates that the two systems are useful platforms to dissect the bases of cell toxicity in the complex biological environment of living organisms, and reinforces the concept that soluble cardiotropic LCs possess intrinsic pathogenicity towards heart cells. Additional models are now needed to recapitulate the full range of features observed in human AL cardiomyopathy, and in particular the process of amyloid formation. Such a model would be a cornerstone to follow the still undefined thread that connects LC proteotoxicity with the presence of fibrils.

REFERENCES:

Animal models of transthyretin amyloidosis to search for FAP patient’s biomarkers

Maria Joao Saraiva

1Instituto de Inovação e Investigação em Saúde (I3S), Universidade do Porto, Portugal; 2 Unidade de Neurobiologia Molecular, Universidade do Porto, Portugal.

ABSTRACT
Novel insights regarding FAP pathogenesis and mechanisms underlying nerve degeneration are paramount for the development of novel therapeutic strategies or disease following biomarkers. Microarray-Based Gene Expression Analysis has been used to search for alterations in the transcriptional machinery in peripheral nerve and dorsal root ganglia of a pre-clinical FAP mouse model carrying the TTR V30M mutation, in a heterozygous Hsf-1 background (Hsf/V30M), presenting TTR non-fibrillar deposition in the PNS. Over and down expressed genes relative to non-transgenic controls were then analyzed following a protocol template that encompasses both the pre-clinical model and human clinical samples. Tissues and plasma samples are investigated by RNA and protein to confirm differential expression of the markers found, their location and co-relation with deposition determined. Since treatment of Hsf/V30M mice with Anakinra or TTR siRNA prevents TTR non-fibrillar deposition in the PNS either by decreasing inflammation or silencing liver TTR synthesis, respectively, the above described analyses are repeated upon treatment and compared.

Following ALL these criteria altered expression of extracellular matrix genes was found. Matrix metalloproteases (MMPs) are endopeptidases, identified as matrix-degrading enzymes that regulate fundamental biological process for normal growth, development and repair. Additionally, robust association with AXON GUIDANCE molecules that account for nerve growth cone formation was also strong.

This approach is the basis for a long term project of several signature biomarkers in patients carrying different TTR mutations and in the follow up of current and future therapies for FAP.
Drosophila melanogaster as a model in protein aggregation diseases

Gunilla T Westermark

Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden

The relevance of flies as model systems for studies of human diseases can be argued, but it should be remembered that several cellular systems e.g. autophagy and toll-like receptor were first identified in Drosophila melanogaster. By using the expression system UAS-GAL4 the expression of one or more genes can be directed and limited to a specific tissue or cell. Effects of transgene expression can be studied alone or in combination or with the use of one or more of the available reporter genes. Also, there are several RNAi libraries available for reduction of endogenous protein expression. It takes less than 1 month to cross and produce new fly-lines and both larvae and adult flies can be studied.

References
Exploring the mechanism of cardiotoxicity in AL amyloidosis: the C. elegans model

P Rognoni1, L Diomede2, M Romeo2, F Lavatelli1, A di Fonzo1, G Palladini1, L Verga3, F Fiordaliso2, RA Cherny4, V Perfetti5, M Salmona2, G Merlini1

1Amyloidosis Research and Treatment Center, Foundation IRCCS Policlinico San Matteo, Pavia, Italy, 2IRCCS-Istituto di Ricerche Farmacologiche “Mario Negri”, Milan, Italy, 3Pathologic Unit, Foundation IRCCS Policlinico San Matteo, Pavia, Italy, 4The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Royal Pde, Parkville, VIC Australia and Prana Biotechnology Ltd., 369 Royal Pde, Parkville, VIC, Australia; 5Medical Oncology Unit, Foundation IRCCS Policlinico San Matteo, Pavia, Italy.

paola.rognoni@unipv.it

INTRODUCTION: In AL amyloidosis, prognosis is determined by the severity of heart involvement. The elucidation of the molecular mechanisms of LC-induced cardiotoxicity is crucial for understanding the pathogenesis of the disease and to develop novel efficacious therapies.

Taking advantage of the knowledge that the Caenorhabditis elegans’ pharynx is considered an orthologue to the vertebrate heart1, we demonstrated that this animal is an effective model for investigating the pathogenic effects of cardiotoxic LC in vivo2.

MATERIAL & METHODS: Amyloidogenic LC with different organ tropism and non-amyloidogenic LC from multiple myeloma subjects, used as controls, were purified from body fluids (urine, serum) of patients with different clinical features, or produced as recombinant proteins3. The cardiotoxicity of LC were assessed in each patient from clinical, histological, biochemical and instrumental parameters4. The effect on C. elegans’ pharynx behaviour of soluble amyloidogenic LC with different organ tropism was investigated on age-syncronized N2 ancestral nematodes.

RESULTS: Exploring the effect of administration to C. elegans of amyloidogenic LC with different organ tropism, we showed that only monoclonal LC isolated from patients suffering from amyloid cardiomyopathy were specifically recognized as toxic by the nematode, impairing its pharyngeal contraction2. This functional alteration was dose-dependent and resulted in persistent pharyngeal dysfunction accompanied to ROS increase, causing ultrastructural alteration in worms’ pharynx and a mitochondrial damage similar to that observed in human hearts of cardiac AL patients. We observed that metal ions have a causal role in promoting oxidative stress, since metal-binding compounds such as 8-hydroxyquinoline and its derivative PBT2 counteracted the LC-induced functional and ultrastructural alterations. In addition, we showed that cardiotoxic LC activate the FOXO/DAF-16 pathway and the expression of proteins involved in stress resistance and survival.

DISCUSSION & CONCLUSIONS: Our findings indicate that Caenorhabditis elegans is a valuable animal model to investigate the molecular mechanisms of cardiotoxicity in AL amyloidosis. Further investigations, including proteomic profiling studies and the generation of transgenic C. elegans strains expressing monoclonal cardiotoxic LC are ongoing.

REFERENCES:
Efficiency of siRNA for removal of transthyretin V30M in a TTR leptomeningeal animal model

P Gonçalves¹,², H Martins¹,², S Costelha¹,², MJ Saraiva¹,²

¹Instituto de Inovação e Investigação em Saúde (I3S), Universidade do Porto, Portugal; ²Unidade de Neurobiologia Molecular, Universidade do Porto, Portugal.

INTRODUCTION: Some TTR mutants target the central nervous system (CNS). FAP with leptomeningeal involvement has been described in 9% of TTR mutations and in V30M patients. These individuals present dementia, ataxia, brain haemorrhages and focal neurological episodes (FNES). FNES occurred in V30M FAP patients with longer disease duration or who have undergone liver transplant to remove the source of plasma mutant TTR as a form of treatment. It is thus to expect that as better treatments for FAP emerge and prolong survival ages, leptomeningeal-vascular CNS deposition will increase and needs special therapies. Deposition in the brain could result from local TTR production in the choroid plexus. Recently, we detected TTR meningeal-vascular deposition in a V30M TTR transgenic mice opening new avenues of research to investigate selective treatments of this condition. TTR siRNA therapeutics remodels the extracellular matrix and enhances clearance of TTR non-fibrillar deposits, suggesting that ECM turnover and TTR clearance might be closely associated and that acting on both mechanisms together may potentiate treatment.

MATERIAL & METHODS: We performed intravascular TTR siRNA injections and appropriate control to investigate: (i) the effect on TTR levels in cerebrospinal fluid; (ii) the efficiency on deposit removal on meningeal and vascular brain blood vessels.

RESULTS: TTR siRNA drastically lowered total TTR blood levels, but had no effect on CSF total TTR levels. Furthermore, it removed TTR deposition in both meninges and in extracellular matrix of brain blood vessels. Brain endothelial cells had no deposition.

DISCUSSION & CONCLUSIONS: Peripheral TTR siRNA administration may result for treatment of leptomeningeal deposition in TTR amyloidoses.
Serum amyloid A in the treatment of sepsis in a mouse model

RP Linke1, A Meinel1, JP Chalcroft2

1 Reference Centre of Amyloid Diseases, Martinsried, Germany, 2 Max Planck Institute of Neurobiology, Martinsried, Germany.
linke@amymed.de

INTRODUCTION:
Severe inflammation with fatal outcome owing to sepsis is a major cause of mortality worldwide. Some 23,000 people are killed every year in the USA alone by infections caused by drug-resistant bacteria. Overuse of antibiotics in people and animals is considered to be the leading cause of multi-resistance. Therefore, sepsis from bacterial invasion has become a major health problem, with an increase of 5-8% fatalities per year, resulting from a lack of normal anti-inflammatory defensive responses in patients. Inflammations, injuries and tumors can lead to a severe liver-induced shift in the concentration of certain blood proteins, the “acute-phase proteins” (APPs). This acute phase reaction is highly complex and not well understood in terms of the individual functional contributions of each protein within the whole reaction. The most prominent acute phase proteins in blood are serum amyloid A protein (SAA) and C-reactive protein (CRP), which are the primary clinical markers of the severity of the acute phase reaction.

MATERIAL & METHODS:
The roles played by both proteins have been examined here in a murine polymicrobial sepsis model (induced by needle puncture of the ligated cecum) which was induced in normal mice and also in two recombinant mice (1). In the latter animals either gp130 (the receptor of IL-6 and signal transducer at the liver surface) or STAT3 (the activator of transcription) was deleted. The severity of sepsis was measured in terms of days-of-survival.

RESULTS:
Sepsis severity was aggravated in gp130 deleted mice, nevertheless, intraperitoneal injection of recombinant human SAA could reduce the severity of sepsis to that found in normal mice. In the case of STAT3-deficient mice, injection of SAA also showed the same beneficial effect. Thus, SAA could terminate the severity of sepsis in both cases. Conversely, when monoclonal antibodies against human AA (mc4/mc29) were added to the protective SAA in solution the protective effect of SAA was deleted. The same reduction of the murine SAA-function was shown with these monoclonals in the murine sepsis model of genetically normal mice, showing that even in the face of the full acute phase reaction the murine SAA function is still acting as a primary acute phase protein within the highly complex acute phase reaction. Moreover in the presence of SAA during the acute phase, myeloid-derived suppressor cells (mainly known for their anti-inflammatory properties in cancer) are mobilized in mice; an effect which could be instrumental in reducing the bacterial load.

DISCUSSION & CONCLUSIONS:
We have shown here that injection of human SAA can greatly reduce the severity of sepsis in mice suffering gp130 deletion or STAT3 deletion. Blocking the beneficial effect of SAA with immuno-specific antibodies indicates that the protective effect is a property of SAA alone. These experiments indicate the value of SAA injection for the reduction of sepsis severity.

REFERENCES:
The 6-minute walk test in AL amyloidosis patients: a single center experience

V Pulido¹, JL Berk², V Sanchorawala²

¹Department of Medicine, ²Amyloidosis Center, Boston University School of Medicine and Boston Medical Center, Boston, MA

vina.pulido@bmc.org

INTRODUCTION: The 6-minute walk test (6MWT) has been widely used as an objective evaluation of functional exercise capacity and response to medical intervention in cardiopulmonary patients. The 6MWT is currently being used as an outcome measure in randomized controlled trials of new therapies for systemic light chain (AL) amyloidosis including the VITAL Amyloidosis phase 3 study of NEOD001, an immunoglobulin G1 antibody against amyloid fibrils. However, little is known about the 6MWT in this specific patient population.

MATERIAL & METHODS: We performed a retrospective study of 120 adults with AL systemic amyloidosis (60 with cardiac involvement and 60 without cardiac involvement) who had their initial evaluation at the Amyloidosis Center of Boston University School of Medicine between 2013 and 2015. All patients were referred for the 6MWT as a measure of functional exercise capacity prior to initiation of therapy. Additional baseline assessments included New York Heart Association (NYHA) class, B-type natriuretic peptide (BNP), troponin I, left ventricular ejection fraction (LVEF), and interventricular septal end diastole thickness (IVSd).

RESULTS: Forty-seven AL amyloidosis patients with cardiac involvement and 41 AL amyloidosis patients without cardiac involvement were included in the final analysis. The mean ages were 59 and 60 years, respectively. The 6-minute walk distances (6MWD) were 368 + 105 meters (mean + SD) and 420 + 116 meters, respectively (p=0.03). Among AL amyloidosis patients with cardiac involvement, the 6MWD differed significantly by NYHA classes. The mean 6MWD was 426 + 91 meters in NYHA Class I patients (n=16) versus 370 + 70 meters in NYHA Class II patients (n=21) versus 273 + 125 meters in NYHA Class III patients (n=10) (p<0.001). No NYHA Class IV patients were included in the analyses, as they were too debilitated to perform the 6MWT.

DISCUSSION & CONCLUSIONS: The 6MWT is a valuable tool in assessing functional exercise capacity in AL amyloidosis patients. In cardiac rehabilitation programs, an improvement of 25 meters on 6MWT is considered the minimal clinically important difference. In this experience, the mean difference in the 6MWT between patients with and without cardiac involvement from AL amyloidosis was 52 meters. Among the AL amyloidosis patients with cardiac involvement, 6MWT correlates with NYHA class, a validated subjective measure of functional exercise capacity.


Table 1. Baseline characteristics and 6MWT results

<table>
<thead>
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<th>Characteristic</th>
<th>Cardiac Involvement</th>
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<tr>
<td>n</td>
<td>47</td>
<td>41</td>
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</tr>
<tr>
<td>λ Light chain (%)</td>
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<td>28 (68)</td>
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<tr>
<td>κ Light chain (%)</td>
<td>7 (15)</td>
<td>13 (32)</td>
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<td>695±786</td>
<td>96±176</td>
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<td>Mean troponin I, ng/mL±SD</td>
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<td>0.016±0.034</td>
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<td>53±23</td>
<td>63±5</td>
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<td>14±2</td>
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<td>Mean 6MWD, m±SD</td>
<td>368±105</td>
<td>420±116</td>
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This project was supported by the Amyloid Research Fund at Boston University
Characteristics and Outcomes in 179 Patients with Advanced Cardiac (stage IIIb) AL Amyloidosis from the ALchemy study cohort

AD Wechalekar¹, B Sevillano¹, D Foard¹, T Lane¹, C Whelan¹, M Fontana¹, S Mahmood¹, S Sachchithanantham, HJ Lachmann¹, JD Gillmore¹ and PN Hawkins¹

National Amyloidosis Centre, Division of Medicine, University College London, London
a.wechalekar@ucl.ac.uk

Introduction

The prognosis of systemic light chain amyloidosis is determined by extent of cardiac involvement. The Mayo cardiac staging system (Dispenzieri et al JCO 2004) is widely used for assessing prognosis. We defined a particularly poor prognostic subgroup (a median survival of 4 months) within Mayo stage III patients characterized by NT-proBNP >8500 ng/L; now termed as stage IIIb disease (Wechalekar et al Blood, 121(17), 2013). Such patients are excluded from clinical trials and treatment outcomes in this patient group are unclear. We report here the characteristics and treatment outcomes of all patients with stage IIIb cardiac AL amyloidosis followed prospectively in the ALchemy study

Patients and Methods.

ALchemy is a prospective observational study of all patients with systemic AL amyloidosis undergoing chemotherapy followed up at the UK National Amyloidosis Centre. All patients with Mayo stage IIIb disease (defined as NT-proBNP >8500 ng/L and cardiac hs-Troponin T >0.05 μg/L) from the first 1000 patients recruited into the study (from 2009 to Jan 2015) are reported here. Patients were treated according to nationally agreed protocols. Organ involvement and hematologic/amyloidotic organ responses were assessed according to 2010 amyloidosis consensus criteria. The primary outcome measure was overall survival (OS) and impact of hematological response on survival.

Results

A total of 179 patients were included. The median age was 65 yrs, 77 (43 %) were female and 102 (57 %) male. All patients had cardiac involvement. Renal involvement was present in 131 (73.2 %) and liver involvement in 28 (15.6 %). The median NT-proBNP was 19056 ng/L (range 8500 –70084 ng/L), median left ventricular (LV) wall thickness was 14.2 mm (range 11-22 mm) and median ejection fraction (EF) was 48.7 % (23-75 %). 34 % were NYHA ≥ grade 3 and 16.8 % had ECOG performance status ≥3. Thirty (17 %) patients died prior starting chemotherapy. Initial treatment regimens were: Bortezomib combinations in 87 (48 %); Thalidomide or Lenalidomide combinations in 51 (28%), alkylator based regimens in 9 (5 %) and rituximab based in 2 (1 %). The hematological responses on an intention to treat basis were: complete response (CR) – 35 (20%), very good partial response (VGPR) 25 (13%), partial response (PR) 32 (18%) and no response (NR) 87 (47%) (NR included patients who died before treatment initiation). The median overall survival (OS) for the cohort was 6 months. Univariate and ROC analysis identified LVEF >55%, dFLC <400 mg/L and SBP >110 mm Hg best predictors of OS. The median OS was significantly better for patients with LVEF>55% (13 months); for dFLC <400 mg/L was 7 months (vs. 3 months for dFLC >400 mg/L); and for those with SBP > 110 mmHg was 10 months (vs. 5 months in those with lower SBP). Median OS for patients achieving a CR/VGPR at day 30 was 26 months compared to 5 months for patients with <VGPR at that time. The median OS for patients who finally achieved CR/VGPR was 38 months, PR 7 months and NR was 2.6 months (log rank p<0.0001). In a multivariate model, LVEF <55% (HR1.5), dFLC >400 mg/L (HR 1.3), SBP <110 mm of Hg (HR 1.5) and not achieving a hematological CR/VGPR (HR 5.3) were independent predictors of poorer outcomes.

Conclusions:

The survival of patients with stage IIIb AL amyloidosis is poor although the current cohort has better outcomes than previous reports. Poor LV systolic function, low SBP and a high clonal burden were associated with particularly poor outcomes. Just over half of all patients achieve a haematological response including a third reaching the goal of VGPR or better associated with strikingly improved outcomes especially for early responders. Although half of all patients will achieve a haematological response and have improved survival, a similar number die due to disease related complications. The former patient group, perhaps, questioning the widely used practice of treating such patients dose reduced chemotherapy which delays clonal responses and the latter raising an urgent need for using therapies that accelerate amyloid removal. We hope that this data will encourage prospective clinical trials in this patient group using rapidly effective chemotherapy regimens in combination of immunotherapy accelerating amyloid fibril clearance.
Evaluation of therapeutic oligonucleotides for familial amyloid polyneuropathy in a stem cell-based in vitro model

C Niemietz¹, V Sauer¹, J Stella¹, G Chandhok¹, S Guttmann¹, Y Avsar¹, S Guo², EJ Ackermann², J Gollo³, BP Monia², A Zibert¹, HH Schmidt¹

¹Klinik für Transplantationsmedizin, Universitätsklinikum Münster, Münster, Germany.
²Ionis Pharmaceuticals, Inc., Carlsbad, CA, USA.
³Alnylam Pharmaceuticals, Inc., Cambridge, MA, USA.

hepar@ukmuenster.de

INTRODUCTION: Familial amyloid polyneuropathy (FAP) is caused by mutations of the transthyretin (TTR) gene. TTR is secreted into the blood, predominantly by the liver. The tetramer can undergo dissociation resulting in extracellular tissue deposition of TTR, followed by dysfunctions in target tissues. The effects of an antisense oligonucleotide (ASO; IONIS-TTRRx) and small interfering RNA (siRNA; ALN-TTR-02) on TTR synthesis are currently being evaluated in phase II/III clinical trials in patients with FAP. Primary hepatocytes from FAP patients are rarely available for molecular analysis and commercial tissue culture cells do not have the genetic background of individual patients.

MATERIAL & METHODS: Hepatocyte like cells (HLCs) were generated from five FAP patients using induced pluripotent stem cells (iPSC). Patients had different TTR mutations (Val30Met, Gly47Ala, Arg34Thr) and showed different phenotypes. Differentiation toward HLCs was achieved using a 3-step in-house protocol with a total cultivation time of 14 days. HLCs were characterized by analysis of typical hepatic markers via RT-PCR, immunocytochemistry, flow cytometry, and functional activity. To assess TTR gene silencing, IONIS-TTRRx and ALN-TTR-02 were introduced into HLCs via transfection.

RESULTS: HLCs of FAP patients could be obtained that showed typical markers of human hepatocytes as assessed by RT-PCR, immunostaining, flow cytometry, and functional analysis. TTR mRNA expression was almost identical to human hepatic cells. A significant downregulation (>80%) of TTR mRNA was induced in the HLCs by both therapeutic oligonucleotides. TTR protein present in the cell culture supernatant of HLCs was similarly downregulated by both oligonucleotides as determined by ELISA and Western blot analysis. Gene expression of other hepatic markers was not affected by the therapeutic oligonucleotides.

DISCUSSION & CONCLUSIONS: IONIS-TTRRx and ALN-TTR-02 were shown to be highly efficient in downregulation of TTR in human hepatic like cells obtained from FAP patients. The use of patient-specific cells derived from induced pluripotent stem cells represents an excellent approach to molecularly assess the efficacy and specificity of novel compounds in target cells that have the individual genetic background of patients.
Treatment of transthyretin cardiomyopathy with a TTR specific antisense oligonucleotide (IONIS-TTRRx)

MD Benson1, EJ Ackermann2, BP Monia2

1Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, Indiana USA, 2IONIS Pharmaceuticals, Carlsbad, California USA

mdbenson@iupui.edu

INTRODUCTION: ATTR (transthyretin amyloidosis) is an autosomal dominant disease most frequently characterized by polyneuropathy and cardiomyopathy. While peripheral neuropathy is often the most significant clinical manifestation of the disease, restrictive cardiomyopathy is a principal cause of death either from progressive congestive heart failure or cardiac arrhythmia. While treatments with TTR stabilizing agents have shown efficacy in slowing progression of neuropathy, no treatments for altering progression of cardiomyopathy have been reported.

MATERIAL & METHODS: At present, pharmaceutical studies to inhibit the hepatic synthesis of TTR with either TTR specific antisense oligonucleotides or siRNA are ongoing for patients with ATTR neuropathy. In addition, we have instituted a study to evaluate the safety of a TTR specific ASO (IONIS-TTRRx) in patients with moderate to severe amyloid cardiomyopathy. Twenty subjects have been admitted to study after documentation of biopsy proven ATTR and left ventricular wall thickness on echocardiogram of 1.3 centimeters or greater. Safety parameters are monitored over a 24-month period. Echocardiograms are conducted every six months and cardiac MRI is obtained every 12-months when feasible. The study drug IONIS-TTRRx, previously shown to produce substantial reductions in TTR levels, is administered as 300 mg subcutaneously on a weekly basis. Hematology, hepatic, and renal parameters are monitored on a frequent basis.

RESULTS: Twenty patients have been admitted to this study (8 familial ATTR and 12 wild type ATTR). Six subjects have completed greater than one year on the study. Fifteen subjects have completed six months. One subject left the study before six months to receive a heart transplant. Echo evaluations, including left ventricular wall thickness and global systolic strain at 6- and 12-months indicate lack of disease progression in a majority of patients relative to natural history data. This is supported by cardiac MRI studies for five subjects treated for 12-months with a mean decrease in left ventricular mass (LVM) of approximately 5%. IONIS-TTRRx has been well tolerated with no drug-related serious adverse events (SAEs).

DISCUSSION & CONCLUSIONS: IONIS-TTRRx is well tolerated in patients with moderate to severe ATTR cardiomyopathy. Preliminary data showing lack of progression of amyloid deposition as monitored by echocardiography and cardiac MRI are encouraging.
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DETECTION OF CIRCULATING MISFOLDED TTR OLIGOMERS IN TTR POLYNEUROPATHY AND CARDIOMYOPATHY PATIENTS AND A REDUCTION IN LEVELS UPON TAFAMIDIS TREATMENT
Joseph D. Schonhoft, Cecilia Monteiro, Yvonne S. Eisele and Jeffery W. Kelly
The Scripps Research Institute

ABSTRACT
We developed an Ex Vivo plasma approach to quantify circulating misfolded TTR oligomers in both polyneuropathy and cardiomyopathy patients and have demonstrated that the misfolded TTR oligomer concentration dramatically decreases upon Tafamidis treatment.

REFERENCES:
The XV International Symposium on Amyloidosis
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Thioflavin T: Not an all-rounder, but a trustworthy friend for over 27 years
Hironobu Naiki

Department of Molecular Pathology, Faculty of Medical Sciences, University of Fukui, Fukui 910-1193, Japan

ABSTRACT

The detailed in vitro analysis of the molecular mechanisms of amyloid fibril formation is indispensable to elucidate the molecular pathogenesis of amyloidosis. In 1989, we developed a novel fluorometric method to identify amyloid fibrils in vitro based on the unique characteristics of thioflavin T (ThT). Using fluorescence spectroscopy, we found that although ThT itself emits no significant fluorescence when it is excited at 450 nm, it fluoresces brightly at 490 nm after it binds to amyloid fibrils. Since our first report, ThT has become one of the most widely used “gold standards” for selectively identifying amyloid fibrils both in vivo and in vitro. Since the group of University of Pittsburgh developed a lipophilic ThT analog, Pittsburgh Compound B (PiB) as a potential amyloid-imaging PET tracer, PiB is widely used for the diagnosis of Alzheimer’s disease.

The ThT assay system has several advantages. First, under a constant ThT concentration, the fluorescence change is quite linear over a wide range of the weight concentration of amyloid fibrils. Based on this property of ThT, we precisely investigated the polymerization kinetics of murine AApoA-II amyloid fibrils in vitro. In the 1990’s Oslo symposium, we presented that amyloid fibril formation can be explained by a first-order kinetic model: that is, extension of amyloid fibrils proceeds via the consecutive association of precursor proteins onto the ends of existing fibrils. Second, ThT does not practically affect the kinetics of amyloid fibril formation in vitro. Based on this property, ThT is generally included in the reaction mixture to perform the high-throughput monitoring of aggregation kinetics by e.g., an ELISA plate reader. We also visualized seed-dependent amyloid fibril growth of Aβ(1-40) in real-time at the single fibril level using total internal reflection fluorescence microscopy.

There are several pitfalls of ThT assay system. First, ThT does not recognize amyloid fibrils per se, but binds to the molecular groove found typically on the surface of amyloid fibrils and becomes activated as a result of the rotation around a single C-C bond in the middle of the molecule. Actually, the ThT fluorescence can also be evoked by DNA, cyclodextrin, polymer membranes, SDS micelles and porous silicon. Thus, to identify amyloid fibrils in vitro, we should carefully use this system together with other biochemical methods, e.g., turbidity measurement, EM, AFM, CD and ATR-FTIR. Second, the affinity of ThT for many types of amyloid fibrils and the fluorescence intensity per unit weight are different from each other. Thus, to compare the ThT fluorescence between different types of amyloid fibrils, we should perform extensive control experiments. Third, the ThT system cannot fully detect the early aggregates (i.e., oligomers and protofibrils) in the fibril forming pathway. Generally, when amyloid precursor proteins are incubated in vitro, ThT fluorescence kinetics shows a sigmoidal curve. During the initial lag phase, oligomers, protofibrils and fibril seeds are formed. Thus, to precisely analyze the early aggregation pathway of amyloid fibril formation, various biochemical and biophysical methods (e.g., SEC, DLS) should be used. Finally, we should be extremely careful when we evaluate the effects of organic compounds on the amyloid fibril formation, because some compounds compete with ThT to bind to the surface of amyloid fibrils, leading to the reduction in ThT fluorescence.

In this session, we will discuss how to apply the ThT assay system safely to the future amyloid studies.

REFERENCES:
Seed-dependent templating of murine AA amyloidosis

S Nyström¹, A Vahdat shariat panahi ², KPR Nilsson¹, P Westermark ³, GT Westermark⁴, P Hammarström¹, K Lundmark²

¹IFM-Chemistry, Linköping University, Linköping, Sweden. ²Department of Clinical Pathology and Clinical Genetics, and Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden. ³ Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden. ⁴Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden

sofny@ifm.liu.se

INTRODUCTION: The processes of amyloid formation, deposition and rearrangement can be studied both in vitro and in vivo in animal models of disease. When studying these processes using conformation sensitive luminescent conjugated oligothiophenes (LCOs) in genetic mouse models of Alzheimer’s disease (AD) we have shown that the conformational properties of the amyloid changes over time [1]. AA amyloidosis can be induced in outbred mice through seeding with Amyloid Enhancing Factor (AEF) i.e. AA amyloid fibrils purified from tissue of amyloidotic mice [2]. This rapid and robust model of AA amyloidosis allows for detailed studies of the amyloid formation process of this disease and, in a broader perspective an increase in the general understanding of amyloid formation.

MATERIAL & METHODS: Two different batches of AEF were prepared from different pools of animals at different timepoints but using the same protocol. Amyloidosis was induced in NMRI mice by treatment with AEF and AgNO₃ at an age of 6-8 weeks and additional AgNO₃ was injected at 7, 14, 21 days post induction. Mice were sacrificed between 2 and 23 days to establish a kinetic time line of amyloid fibril formation. Congo red and LCO staining was performed on tissue sections to establish amyloid load and conformational variability. Biochemical experiments were performed on the inocula to deduce differences in fibril morphology and composition.

RESULTS: The two different AEF preparations, AEF 1 and AEF 2, displayed significantly different LCO spectral signatures indicating differences in conformation. The two AEF preparations were used to induce amyloid in two sets of mice in identical experiments. LCO analysis of tissue from mice sacrificed at different time points post induction showed that the AEF 1 with higher qFTAA fluorescence provoked a more rapid increase of qFTAA positive amyloid in both spleen and liver than AEF 2 that displayed no or very low qFTAA fluorescence and then only at very late time points. Congo red grading of spleen also revealed a denser amyloid load at earlier time points in mice induced with AEF 1.

DISCUSSION & CONCLUSIONS: Rearrangement of amyloid over time has been shown by us and others [1, 3] in the case of Aβ peptide in vitro and in vivo. We now display a similar phenomenon in an unrelated amyloid system, demonstrating that this could be a generic property for many if not all amyloidogenic proteins. The results also confirm our previous findings of prion-like transmission of AA amyloid in vivo [4] and shows that the strain dependent conformation templating established for prion disease can also be applied to AA amyloid in animal models.

FRET-based observation of intracellular SAA fibril formation

S Claus1*, K Meinhardt1*, T Aumüller1, C Haupt1, P Walther2, T Syrovets3, T Simmet3, M Fändrich1

1 Institute of Protein Biochemistry, Ulm University, Ulm, Germany. 2 Central Electron Microscopy Facility, Ulm University, Ulm, Germany. 3 Institute of Pharmacology of Natural Products & Clinical Pharmacology, Ulm University, Ulm, Germany.

* authors contributed equally

stephanie.claus@uni-ulm.de

INTRODUCTION: Long-standing evidence shows the presence of AA fibrils in lysosomes of histological sections obtained from animal amyloidotic tissue1. However, whether fibril formation starts intracellularly or fibrils were internalized by the cells to induce their degradation is so far unclear.

Here we established a cell based model to show the intracellular fibril formation of internalized serum amyloid A (SAA) protein.

MATERIAL & METHODS: J774A.1 cell culture, confocal live cell microscopy, flow cytometric analysis, fluorescent protein modification, Förster resonance energy transfer (FRET), cell viability assays, inhibitory studies, scanning electron microscopy (SEM)

RESULTS: Here we show that J774A.1 cells incubated with high density lipoprotein (HDL) and murine SAA1.1 (HDL-SAA) internalize the SAA within 5 h. Confocal microscopic images confirm the intracellular localization of SAA. Incubating cells with LysoTracker® and HDL-SAA shows that internalized SAA is accumulated within lysosomes. By using inhibitors blocking the uptake of SAA, we demonstrate that SAA is internalized through clathrin-dependent endocytosis, phagocytosis or pinocytosis. Uptake pathways were further confirmed with colocalization of internalized SAA and transferrin, pHrodo™ Red Zymosan A BioParticles® or lucifer yellow.

We demonstrate that SAA fibril formation starts intracellularly after internalization of SAA. Therefore, we incubated cells with HDL-SAA including two SAA variants fluorescently labelled with Alexa Fluor® 488 or Alexa Fluor® 594 at cysteine residue 101. Observation of FRET signals inside cells after 24 h, but not after 4 h, indicates that SAA1-Cys-AF488 and SAA1-CysAF594 are in close spatial proximity and have integrated into the same fibril.

Intracellular fibril formation is toxic to the cells, leads to lysosomal leakage as demonstrated by acridine orange and extracellular deposition of AA fibrils after several days. Apoptosis was observed after 24 h incubation as an enhanced caspase 3/7 activity and an increase of cellular DNA fragmentation determined by the TUNEL assay. Reduction of cell viability measured by MTT assay and extensive fragmentation of the plasma membrane after 6 days of incubation further supported the observation of cellular death.

DISCUSSION & CONCLUSION: Our data imply that fibril formation starts within an intracellular vesicular compartment into which the protein is internalized through clathrin-dependent endocytosis, phagocytosis or pinocytosis. Having established a robust system we can link it to other types of amyloid diseases as there has been evidence for an association of macrophages, such as AL amyloidosis2 and we are now able to identify factors modulating fibril formation in cells.

First report of $MYD88^L265P$ somatic mutation in IgM-associated light chain amyloidosis

R Chakraborty$^1$, A J Novak$^1$, S M Ansell$^1$, E Muchtar$^1$, P Kapoor$^1$, S R Hayman$^1$, A Dispenzieri$^1$, F K Buadi$^1$, M Q Lacy$^1$, R L King$^2$, M A Gertz$^1$

Chakraborty.Rajshekhar@mayo.edu

1. Division of Hematology, Mayo Clinic, Rochester, Minnesota.
2. Division of Hematopathology, Mayo Clinic, Rochester, Minnesota.

INTRODUCTION: A mutation in myeloid differentiation factor gene, $MYD88$, leading to constitutive activation of the nuclear factor (NF)-κB pathway was initially shown to be oncogenically active and highly recurrent in Waldenström’s Macroglobulinemia (WM) and other B-cell lymphoproliferative disorders (LPDs). Although primary systemic amyloidosis is associated with clonal plasma cell proliferative disorders or B-cell LPDs, the frequency of an activating $MYD88$ mutation has not been studied in this population so far. The objective of our study was to detect the frequency of activating $MYD88$ mutation in IgM-associated light chain amyloidosis cases and identify clinicopathologic correlations.

MATERIAL & METHODS: Bone marrow specimens were obtained from 14 patients with available archival tissue after approval by the Mayo Clinic Institutional Review Board. All samples were examined by standard morphology and flow cytometric evaluation of B-cells and plasma cells compartments. DNA was extracted from CD138$^+$ sorted cells isolated from the bone marrows of IgM amyloidosis patients using the Gentra PureGene DNA Isolation Kit (Qiagen, Valencia, CA, USA). Real-time allele-specific oligonucleotide PCR (ASO-PCR) was performed using qBiomarker Somatic Mutation Assay for $MYD88^85940$ (SABiosciences, Qiagene, Hilden, Germany) according to the manufacturer’s protocol. The RT-PCR reaction was analyzed using CFX96 real-time thermal cycler (Bio-Rad, Hercules, CA, USA). To obtain a ΔΔCt range for wild-type alleles, the assay was performed on DNA from 10 $MYD88^WT$ controls. The cut-off for wild-type versus mutant $MYD88$ was a ΔΔCt value of 0.003.

RESULTS: Median age of the study population was 60 years (range 48-70 years). Ten out of 14 patients (71%) patients were positive for the $MYD88^{L265P}$ mutation. Among $MYD88^{L265P}$ positive patients, bone marrow examination revealed clonal plasma cells in 8 patients, clonal B-lymphocytes in 9 patients and both clonal B-lymphocytes and plasma cells in 7 patients. Of note, 9/10 patients with $MYD88^{L265P}$ had clonal B-lymphocytes in bone marrow, as opposed to 1/4 patient with $MYD88^{WT}$ (WT). Median serum M-spike in $MYD88^{L265P}$ and $MYD88^{WT}$ patients were 1.15 g/dl (range 0.24-2.3 g/dl) and 0.55 g/dl (range 0.1-0.9 g/dl) respectively. Cardiac involvement was seen in 1/10 patients with $MYD88^{L265P}$ and 2/4 patients with $MYD88^{WT}$. Nervous system involvement (peripheral, and/or autonomic) was seen in 4/10 patients with $MYD88^{L265P}$ and 0/4 patients with $MYD88^{WT}$. One $MYD88^{L265P}$ positive patient had multiple cranial nerve palsies, an unusual manifestation of amyloidosis. Estimated median-follow up of surviving patients was 77.9 months (95% confidence interval, 26.3-120.3 months). Median overall survival (OS) and progression-free survival (PFS) from diagnosis for the entire cohort was 95.8 months (95% CI, 41.2-NR) and 48.2 months (95% CI, 27.8-120.3) respectively. Median OS in patients with $MYD88^{L265P}$ and $MYD88^{WT}$ was 83.0 months (95% CI, 11.9-108.6) and NR (95% CI, 16.4-NR) respectively, 5-year OS rate being 60% (95% CI, 24%-87%) and 75% (95% CI, 24%-97%) respectively. Median PFS in patients with $MYD88^{L265P}$ and $MYD88^{WT}$ were 43.3 months (95% CI, 11.9-75.7) and 120.3 (95% CI, 14.0-120.3) respectively.

DISCUSSION & CONCLUSIONS: ASO-PCR has identified highly recurrent $MYD88^{L265P}$ mutations in bone marrow of patients with IgM-associated AL amyloidosis. Patients with $MYD88^{L265P}$ had a lower incidence of cardiac involvement, a higher incidence of neuropathy and a higher incidence of clonal B-lymphocytes in bone marrow, compared to $MYD88^{WT}$. Given the ease of detection of $MYD88^{L265P}$ mutation, further studies should focus on assessing its frequency in larger cohorts of B-cell LPDs and correlating with clinical characteristics, including the development of systemic amyloidosis.
Cell damage in light chain amyloidosi: fibril internalization, toxicity and cell-mediated seeding

M Marin-Argany1, Y Lin2,3, P Misra1, A Williams4, JS Wall4, LR Elsbernd5, M McClure6, M Ramirez-Alvarado1,5

1 Departments of Biochemistry and Molecular Biology; 5 Department of Immunology; 2 Division of Hematology; 3 Division of Transfusion Medicine; 6 Department of Radiology, Mayo Clinic, Rochester, MN, USA. 4 Departments of Medicine and Radiology, The University of Tennessee, Knoxville, TN, USA.

marinargany.marta@mayo.edu

INTRODUCTION: Light chain (AL) amyloidosis is an incurable human disease characterized by the misfolding, aggregation, and systemic deposition of amyloid composed of immunoglobulin light chains (LC). This work describes our studies on potential mechanisms of AL cytotoxicity.

MATERIAL & METHODS: We have studied the internalization of AL soluble proteins and amyloid fibrils into human AC16 cardiomyocytes by using real-time live-cell image analysis.

RESULTS: Our results show how external amyloid aggregates rapidly surround the cells and act as a recruitment point for soluble protein, triggering the amyloid fibril elongation. A fraction of aggregates surrounding the AC16 cells is internalized via macropinocytosis. AL amyloid fibrils are shown to be highly cytotoxic at low concentrations. Additionally, soluble protein internalizes via macropinocytosis into AC16 cells in a size-dependent manner. Caspase assays revealed soluble protein induces apoptosis, demonstrating different cytotoxic mechanisms between soluble protein and amyloid aggregates.

DISCUSSION & CONCLUSIONS: This study emphasizes the complex LC-cell interactions that result in fibril internalization, protein recruitment, and cytotoxicity that may occur in AL amyloidosis.


Fig. 1: Light chain internalization into human cardiomyocytes - Representative images comparing AC16 cells after 24 h of incubation with A) 1 μM OGLC soluble, B) 1 μM OGLC fibrils.
DISCOVERY OF TRANSTHYRETIN STABILIZERS DISPLAYING RESILIENCE TO THE EFFECT OF THE V30M MUTATION

CJV Simões¹, ZL Almeida², BL Victor¹, B Nascimento², DCS Costa², AL Cardoso², MR Almeida⁴, MJ Saraiva⁴, TMVD Pinho-e-Melo², RMM Brito²

¹ BSIM² – Drug Discovery, Cantanhede, Portugal. ² Coimbra Chemistry Centre and Chemistry Department, University of Coimbra, Portugal. ³ Center for Neuroscience and Cell Biology, University of Coimbra, Portugal. ⁴ I3S – Instituto de Investigação e Inovação em Saúde, University of Porto, Portugal. ⁵ Institute for Molecular and Cell Biology, University of Porto, Portugal.

brito@ci.uc.pt

INTRODUCTION: More than 113 mutations in the transthyretin (TTR) gene have been linked to amyloid formation, with the Val30Met (V30M) mutation being the most common in Familial Amyloid Polyneuropathy (FAP) [1]. TTR stabilization by molecules endowed with chaperone-like activity has been proven a viable approach to the treatment of FAP, but the need to identify TTR stabilizers showing high resilience to mutation effects remains a priority. Herein, we present the results of TTR stabilization experiments conducted with three novel series of TTR amyloid inhibitors, and contrast them with those of tafamidis – the first and only drug treatment approved for FAP.

MATERIALS & METHODS: Human plasma TTR stability was evaluated by isoelectric focusing (IEF) in semi-denaturing conditions (ex vivo), as described in reference [2]. 30 μL samples of plasma were incubated with 5 μL of 10 mM solution of test compounds. The samples were then subjected to native PAGE and the gel band containing TTR was excised and applied to an IEF gel. IEF was carried out in presence of 4 M urea, containing 5% (v/v) ampholytes (pH 4-6.5). Staining with Coomassie Blue was followed by densitometry of the gels. The results are expressed as the average ratio of TTR tetramer to total TTR.

RESULTS: As shown in Fig. 1, all compounds hold ability to stabilize TTR to some extent in plasmas from normal subjects. While tafamidis, AT09 and AT50 compounds (best-in-series) showed limited ability to stabilize TTR in plasmas of V30M carriers, respectively with a tetramer to total protein ratio of 0.451, 0.481 and 0.52, the best-in-series AT40 compound yielded a ratio of 0.897.

DISCUSSION & CONCLUSIONS: Compared with other test compounds and tafamidis, our AT40 compound series shows the highest level of stabilization of TTR in human plasma of carriers of the amyloidogenic mutation V30M.


Fig. 1: Ex vivo evaluation of TTR stability in plasmas from WT subjects and TTR V30M carriers by isoelectric focusing (IEF) under semi-denaturating conditions – in absence (vehicle) or presence of test compounds. The bars in the chart represent the average ratio of TTR tetramer to total TTR, obtained by densitometry of the IEF gels for 5 independent samples of WT subjects and TTR V30M carriers.
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Electron tomography reveals the fibril structure and lipid interactions in amyloid deposits

M Kollmer¹, K Meinhardt¹, C Haupt¹, F Liberta¹, M Wulff²,
J Linder¹, C Loos¹, T Syrovets², T Simmet², P Westermark³, GT Westermark⁴, P Walther⁵, M Fändrich¹

¹ Institute of Protein Biochemistry, Ulm University, Ulm, Germany. ² Institute of Pharmacology of Natural Products & Clinical Pharmacology, Ulm, Germany. ³ Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden. ⁴ Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden. ⁵ Central Electron Microscopy Facility, Ulm University, Ulm, Germany.
marius.kollmer@uni-ulm.de

INTRODUCTION: Almost all previous research on the detail structure of amyloid was focused on the properties of individual fibrils, but little is known about fibril organization and morphology within an amyloid deposit. We here use electron tomography to investigate the three dimensional arrangement of the fibrils and their lipid interactions in amyloid deposits formed in the cell culture model of AA amyloidosis [1, 2].

MATERIAL & METHODS: J774A.1 cell culture, scanning electron tomography (300 kV field emission transmission electron microscope), negative stain electron microscopy (120 kV transmission electron microscope), persistence length measurements, cell viability and cellular uptake assays, Thioflavin T and Congo red binding assays, X-ray diffraction measurement, total reflectance Fourier-transform infrared spectroscopy.

RESULTS [3]: The fibrils formed by the cell model exhibit typical amyloid characteristics such as cross-β structure, increased β-sheet content and binding of Congo red or Thioflavin T. They are toxic to neighbouring cells which internalize these fibrils by phagocytosis such that the internalized fibrils accumulate within the lysosomes inducing lysosomal leakage and cell death.

These fibrils form extracellular deposits which we reveal here to consist of at least three different network structures. These structures are termed fibril meshwork, fibril bundle and fibril star, describing the relative orientation of the fibrils. A fibril meshwork consists of fibrils with random orientation, fibril bundles show parallel fibrils while a fibril star is made up of fibrils that apparently radiates out from a central spot.

We further found that the formed amyloid deposits are frequently infiltrated by vesicular lipid inclusions, encompassing tubular networks, spherical structures, electron dense structures and multivesicular assemblies. Fibrils interact with the lipids in two ways: first, via their lateral surface and second, with the tip of the fibril which can distort the lipid bilayer.

DISCUSSION & CONCLUSIONS: Our data show that electron tomography is a powerful technique to illuminate the super-structural organization of fibrils within a deposit and the interaction of these filaments with non-fibril components. The found structures correlate with TEM analyses of histological sections that did not use tomography and were thus unable to reveal the three dimensional deposit architecture.

INTRODUCTION:
In immunoglobulin light chain amyloidosis, secreted antibody light chain variable domains form oligomers and amyloid fibrils. For the development of therapeutic strategies, it is crucial to obtain a mechanistic and structural understanding of the process of oligomer and fibril formation. This also allows to study the modes of interaction between the different conformations of the protein with the drug candidate epigallocatechin gallate (EGCG).

MATERIAL & METHODS:
We use a combination of solid- and solution-state NMR to elucidate the process of fibril formation as well as the structure of the amyloid deposits. For the murine antibody MAK33 light chain variable domain, the progression from native monomers and dimers to oligomers and fibrils can be tightly controlled by choosing appropriate experimental conditions. This enables us to look separately at the individual species. NMR studies of the variable domain with and without EGCG are complemented by docking experiments and transmission electron microscopy.

RESULTS:
We could assign > 40 residues of the light chain variable domain in the rigid parts in the fibril structure, which are likely to constitute the hydrophobic core of the fibrils. These first structural insights are complemented by identification of dynamic regions in fibrils and oligomeric intermediates.

We found two competing modes of interaction between the native monomers and EGCG. While EGCG binds to one defined binding site with a proline residue at its center, there is another binding mode causing precipitation. Solid-state NMR analysis reveals that these precipitates are largely unstructured, in contrast to amyloid fibrils.

DISCUSSION & CONCLUSIONS:
We gained first insights into the topology of both oligomeric intermediates and amyloid fibrils. Future works might benefit from the identification of the fibril core for rationalizing the effects of amyloidogenic mutants or rational drug design.

EGCG can have different effects on light chain variable domains, dependent on sequence and biophysical properties. Amyloidogenic sequences\textsuperscript{1,2} are preferred targets for precipitation by EGCG. Understanding of these effects will help to predict the response to EGCG for individual patients.

REFERENCES:
Novel styrylbenzene derivatives for detecting amyloid deposits: EEEFSB

A Izaki¹, K Obayashi¹, M Nakazono², K Sasamoto¹, K Tomiyoshi¹, G Suenaga⁵, M Ueda⁵, T Yamashita⁵, M Tasaki¹, Y Yanagisawa¹, T Masuda⁵, Y Misumi⁵, Y Ando⁵

¹Department of Morphological and Physiological Sciences, Graduate School of Health Sciences, Kumamoto University, Kumamoto, Japan. ²Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan. ³Dojindo Laboratories, Kumamoto, Japan. ⁴Department of Clinical Radiation Technology, Graduate School of Health Sciences, Kumamoto University, Kumamoto, Japan. ⁵Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan.

konen@kumamoto-u.ac.jp

INTRODUCTION

Various styrylbenzene compounds were synthesized and evaluated as mainly Aβ amyloid sensors. These compounds, however, cannot be used for detecting amyloid deposition in peripheral nerves because of the inherent sensitivity of the compounds. These compounds often generate false positives especially in the basement membrane of blood vessels in histochemical studies. To overcome these problems, we must first synthesize other styryl compounds for detecting amyloid fibrils in tissues.

MATERIALS & METHODS

A wide variety of symmetrical and unsymmetrical styrylbenzene derivatives were synthesized and then these compounds were used to detect amyloid fibrils in autopsy and biopsy samples from patients with various systemic and localized forms of amyloidosis such as transthyretin (TTR)-related familial amyloidotic polyneuropathy (FAP), senile systemic amyloidosis (SSA), amyloid A (AA) amyloidosis, localized AL amyloidosis, and Alzheimer’s disease.

RESULTS

1-Methoxy-2,5-bis-styrylbenzene and 2-(2-(2-fluoroethoxy)ethoxy)ethoxy)-2,5-bis-styrylbenzene (EEFSB) detected amyloid fibrils in both in vitro and in vivo histopathological studies. 1-Methoxy-2,5-bis-styrylbenzene also showed a high strength of fluorescence with amyloid deposition in peripheral nerves in a patient with FAP.

DISCUSSION & CONCLUSIONS

1-Methoxy-2,5-bis-styrylbenzene and EEEFSB may prove a useful tool for diagnosing amyloidosis, not only in a histochemical study but also in whole body amyloid positron emission tomography (PET) imaging.
Identification of amyloid precursor protein from autopsy and biopsy specimens using LMD-LC-MS/MS: the experience in Kumamoto University.

M Tasaki1,2, M Ueda1, K Obayashi2, Y Kinoshita1, S Matsumoto1, M Mizukami1, T Masuda1, Y Misumi1, T Yamashita2, Y Ando1

1Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan. 2Department of Morphological and Physiological Sciences, Graduate School of Health Science, Kumamoto University, Kumamoto, Japan. 3Diagnostic Unit for Amyloidosis, Department of Neurology, Kumamoto University Hospital, Kumamoto, Japan

Tasaki@kumamoto-u.ac.jp

INTRODUCTION:

Recently, laser microdissection (LMD)-liquid chromatography tandem mass spectrometry (LC-MS/MS) has been used to identify an amyloid precursor protein from amyloid-laden formalin-fixed paraffin embedded (FFPE) tissues. We also have used both this system and immunohistochemistry (IHC) to diagnose amyloidosis.

The aim of this study was to evaluate the usefulness of LMD-LC-MS/MS for typing of amyloidosis in Kumamoto University.

MATERIAL & METHODS:

We examined 186 FFPE samples obtained from patients between 2012 and 2016. Sections were stained with Congo Red (CR) and CR-positive areas were microdissected by LMD 7000 (Leica Microsystems). Extracted peptides by trypsin digestion were measured and analyzed by LC-MS/MS LTQ Velos Pro system (Thermo Fisher Scientific). We compared the results with clinical information, including results by IHC.

RESULTS:

LC-MS/MS could detect several amyloid precursor proteins, including immunoglobulin light chain (λ), immunoglobulin light chain (κ), TTR, SAA from FFPE tissue sections. SAP, ApoE and vitronectin were also detected by MS. MS results were almost consistent with clinical diagnosis. In several cases, in which IHC was inconclusive or negative, amyloid precursor protein were detected by MS analysis. Furthermore, MS also identified a novel amyloid precursor protein from FFPE tissue sections.

DISCUSSION & CONCLUSIONS:

Our results suggest LMD-LC-MS is a powerful tool for typing of amyloidosis. Especially, it is effective to identify a novel amyloid precursor protein from FFPE tissues.
Role of C-terminal portion of transthyretin on amyloid formation

M Ueda¹, M Mizuguchi², Y Misumi¹, M Tasaki¹, G Suenaga¹, S Matsumoto¹, M Mizukami¹, T Masuda¹, T Yamashita¹, B Kluve-Beckerman⁴, JJ Liepnieks⁴, MD Benson⁴, Y Ando¹

¹Department of Neurology, Graduate of Medical Sciences, ³Department of Physiology, Graduate School of Health Sciences, Kumamoto University, Kumamoto, Japan, ²Faculty of Pharmaceutical Sciences, University of Toyama, Toyama, Japan, ⁴Department of Pathology and Laboratory Medicine, Indiana University, Indiana, USA

INTRODUCTION

C-terminal fragments of transthyretin (TTR) were reportedly found in amyloid deposits in hereditary and wild-type ATTR amyloidosis. However, pathological roles of the TTR fragments remain to be determined.

METHODS

To elucidate amyloid formation of the full-length and fragments of TTR, recombinant full-length TTRs (wild-type and V30M) and TTR fragments (N-terminal (TTR1-80) and C-terminal portions (TTR49-127 and TTR81-127) ) were employed in this study. Amyloid formation of those TTRs in test tube and in cell culture were analyzed by Thioflavin T assay and cell-based assay, respectively.

RESULTS

Although the C-terminal fragment (TTR81-127) formed amyloid fibrils in PBS, the N-terminal (TTR1-80), another C-terminal fragment (TTR49-127), and full-length TTRs did not form any amyloid fibrils in the same condition. The TTR81-127 also formed amyloid deposits on cultured cells. Amyloid deposits derived from TTR81-127 induced apoptosis in cultured cells.

CONCLUSION

The C-terminal portion (TTR81-127) may play an important role on amyloid formation in hereditary and wild-type ATTR amyloidosis.
Pro-inflammatory S100A9 protein as a hallmark of traumatic brain injury

C Wang, I Iashchishyn, J Kara, I Horvath, R Rofougaran, A Mahadevan, SK Shankar, LA Morozova-Roche

1 Department of Medical Biochemistry and Biophysics, Umeå University, Umeå, Sweden.
2 Human Brain Bank, NIMHANS, Bangalore, India

chao.wang@umu.se

Traumatic brain injury (TBI) is viewed as a risk factor and potentially precursor state for developing neurodegenerative Alzheimer’s and Parkinson’s diseases. TBI is often accompanied by inflammatory process which can play causative role in triggering the amyloid cascade typical for neurodegenerative diseases. Here we conducted systematic studies on the involvement of inflammatory S100A9 protein in TBI. S100A9 acts as a pro-inflammatory mediator and its elevated level was found in many inflammatory conditions, including TBI, inflammation-associated Alzheimer’s disease, cerebral ischemia and others. Recently the abundance of S100A9 mRNA was identified as a strong feature of aging and a novel mechanisms of age-associated inflammation sustained by S100A9 was suggested.

By using immunohistochemistry with a range of antibodies towards S100A9, amyloid fibrils, oligomers, NeuN, and others we have explained 19 brain tissues of TBI and controls with the survival time after the impact from few hours to 1 month. This enabled us to follow the dynamics of biochemical, cellular and morphological changes occurring in post-traumatic tissues. S100A9 was abundantly present in all TBI brain tissues. It formed numerous extracellular inclusions in a form of plaques, which were not yet of amyloid nature. These plaques reduced in quantity with increasing post-traumatic time and nearly completely disappeared after 1 month since trauma, indicating that efficient clearance mechanisms are working in the tissues. We have observed abundant presence of S100A9 within neuronal and microglial cells, forming intracellular inclusions recognised by oligomeric antibodies. Amyloid potential of S100A9 was examined in vitro experiments by using AFM and monitoring its aggregation kinetics by a range of fluorescence dyes such as thioflavin-T, h-FTAA and p-FTAA. We suggest that increased intracellular level of S100A9 and its intracellular but not extracellular aggregation could be linked to the amyloid cascade leading to neurodegeneration.

Acknowledgement. We thank Peter N Nilsson for gift of h-FTAA and p-FTAA dyes.
Mass spectrometric characterization of post-translational modifications of pathological free light chains from bone marrow

K Heinig¹, JT Vanselow², DR Leon³, T Steinbrunn³, RC Bargou⁴, U Holzgrabe¹, A Schlosser², CE Costello³, P Kapková¹

¹Institute of Pharmacy and Food Chemistry, Julius-Maximilians-University Würzburg, Am Hubland, 97074 Würzburg, Germany; ²Rudolf Virchow Center for Experimental Biomedicine, University of Würzburg, Würzburg, Germany; ³Center for Biomedical Mass Spectrometry, Boston University School of Medicine, Boston, MA, USA; ⁴Department of Internal Medicine II, Division of Hematology and Medical Oncology, and Comprehensive Cancer Center Mainfranken, University of Würzburg, Würzburg, Germany

INTRODUCTION:
Monoclonal gammopathy patients suffer from unregulated plasma cell proliferation, which causes an overproduction of immunoglobulins or of their fragments. This work analyses immunoglobulins from patients who suffer from multiple myeloma and from patients with multiple myeloma and amyloid light chain (AL) amyloidosis, two diseases in which free light chains of antibodies (FLC) are excessively produced. Although both multiple myeloma and AL amyloidosis patients show an overproduction of FLCs, extracellular depositions of free light chains in organs are observed only with AL amyloidosis; the deposits cause organ failure and therefore death of the patients. Reasons for the deposition of this protein might be variations in the amino acid sequence or post-translational modifications (PTMs) in the FLCs, coupled with local environmental effects.

MATERIAL & METHODS:
Prediction of the amino acid sequences was performed with mRNA from the plasma cell using FLC-specific primers and PCR technology. For PTM analysis, FLCs were purified with affinity chromatography from bone marrow and analyzed with electrospray-ionization tandem mass spectrometry using electron transfer dissociation (ETD) and higher energy dissociation (HCD) fragmentation on Orbitrap™ instruments.

RESULTS:
In order to increase knowledge regarding the structural characteristics of the free light chains involved in pathological disorders of plasma cells, this study analysed the LC gene and amino acid sequences and the appearance of post-translational modifications in FLCs of the above-mentioned patients. Two-step purification generated clean LC samples. MS results verified the expression of all LC sequences predicted from gene sequencing. Extensive post-translational modifications were detected; each was identified and site-specifically assigned.

DISCUSSION & CONCLUSIONS:
Methylation and S-sulfonation at cysteine and O-HexNAc modifications at serine and threonine in FLCs obtained from bone marrow are reported here for the first time. Other modifications included oxidation and acetylation, which in some cases occur in vivo and in others can be artifacts introduced during sample handling and analysis.

This report demonstrates the first isolation of free light chains from bone marrow and presents the first post-translational modifications found in patients with multiple myeloma, with and without amyloid light chain deposits.

This work was supported by the Fond der Chemischen Industrie (FCI). Bavarian Research Alliance (BayFOR) funded the international research cooperation with the Boston University Center for Biomedical Mass Spectrometry, which is supported by US National Institutes of Health grants P41 GM104603 and S10 RR020946 and contract HHSN268201000031C (to CEC) and the BUSM Amyloid Research Program.
Current concepts to target liver disease

V Sauer¹, C Niemietz¹, HH Schmidt¹

¹Klinik für Transplantationsmedizin, Universitätsklinikum Münster, Münster, Germany.
hepar@ukmuenster.de

Liver has a major role in the human body with numerous functions, including the homeostasis of lipids, glycogen, and ions, decomposition of red blood cells, synthesis of important plasma proteins, hormone production, and first entry level of detoxification. From the estimated ≈10,000 genes present in the human genome directly related to a disease a significant portion is expressed in the liver, highlighting the importance of the organ for the emerging concepts of precision medicine. In addition, the unique localization of the liver within the blood system as well as specialized histological features of its architecture, like the space of Disse, represent attractive targets to assess the efficacy of novel concepts for therapy. Evolving concepts for therapy of liver can be classified to (i) the introduction of therapeutic oligonucleotides, most prominently represented by antisense (ASO) or small interfering RNAs (siRNA), (ii) the gene correction or replacement strategies of severe mendelian conditions, e.g. by clustered regularly interspaced short palindromic repeat (CRISPR), and (iii) stem cell-based approaches that involve the transplantation of in vitro cultured cells or the transfer of genes encoding factors by highly specialized genetic vectors in order to favorably stimulate the regeneration of the diseased status. While some of these approaches are currently entering the clinical phase, important issues have yet to be resolved, including technical hurdles and important ethical considerations. An overview of such approaches in the light of transthyretin-related and other liver diseases will be given.
NOVEL ANTI-MEDIN ANTIBODIES DETECT MEDIN DEPOSITS IN AORTIC ANEURYSM, MARFAN SYNDROME AND OTHER CARDIOVASCULAR DISEASES


Prothena Biosciences Inc, South San Francisco, California, USA.

paul.shughrue@prothena.com

INTRODUCTION: Medin, a 50-amino acid cleavage fragment of MFG-E8/lactadherin, self-assembles into fibrils to form amyloid deposits found in patients with aortic aneurysms. Although the pathogenic nature of these aggregates is not fully understood, it is thought that medin deposition perturbs smooth muscle cell function and affects the integrity of the aortic wall. To better understand the relationship between medin misfolding and vascular pathophysiology, we generated novel, neo-epitope antibodies that are specific to medin and that lack cross-reactivity to the parent MFG-E8 protein.

MATERIAL & METHODS: Mice were immunized with either full-length human medin or a C-terminal fragment of human medin. Hybridomas were cloned, and antibodies were screened for activity using ELISA, Western blot analysis, and immunohistochemistry.

RESULTS: Two antibodies with distinct properties were identified and further characterized. The antibody PRT6B3 bound to the N-terminus region of medin, and, though it cross-reacted with denatured MFG-E8, it did not bind to normally folded protein. The antibody PRT18G1 is a medin-specific antibody that binds to a neo-epitope in the C-terminus region of the peptide. Western blot analysis showed that PRT6B3 and PRT18G1 antibodies immunoreacted with both monomeric and oligomeric forms of recombinant medin. Immunoreactivity for endogenous human medin was assessed immunohistochemically using tissue of patients with aortic aneurysm, Marfan syndrome, or other cardiovascular diseases. Both PRT6B3 and PRT18G1 immunolabeled medin in vascular diseased tissue with distinct intensity and patterns of staining. Although PRT6B3 stained dense aggregated (Thioflavin S+) material or amorphous deposits in and around the aneurysm, PRT18G1 showed little to no staining. In contrast, PRT18G1 widely stained structures in the tunica media of aorta, a region composed of elastin fibers and smooth muscle cells. Further studies with PRT6B3 showed strong staining of all aortic samples (normal, aneurysm, atheroma, atherosclerosis, and hypertension) as well as diseased coronary artery and vena cava. Staining was also seen in smaller vessels in the heart and kidney and in cardiac scar tissue after myocardial infarction. PRT18G1 staining was also seen in normal and diseased aorta at a lower intensity than was seen with PRT6B3. In addition, PRT18G1 stained necrotic areas of cardiac tissue after acute myocardial infarction, and it stained the myocardial scar tissue. Additional staining was observed in vein thrombosis and varicose vein tissue. Interestingly, this antibody labeled a wide variety of granulation tissue derived from the groin, intestine, ovary, abdominal wall, and stomach.

DISCUSSION & CONCLUSIONS: Our data demonstrated that novel anti-medin antibodies immunoreact with deposits in tissue samples from patients with aneurysm, Marfan syndrome, and other cardiovascular diseases. The differential distribution of immunolabeling seen with anti-medin antibodies might represent specific reactivity to different aggregation assemblies of medin within the diseased vasculature.
NOVEL CONFORMATION-SPECIFIC MONOCLONAL ANTIBODIES AGAINST AMYLOIDOGENIC FORMS OF TRANSTHYRETIN BIND SPECIFICALLY TO TTR DEPOSITS PRESENT IN DISEASE TISSUE DERIVED FROM ATTR AMYLODOSIS PATIENTS

JN Higaki1, NJ Galant2, KC Hadley2, A Won1, SJ Tam1, K Flanagan1, T Nijjar1, R Torres1, JR Tapia1, J Salmans1, R Barbour1, A Chakrabartty2, CM Yip3, W Zago1, GG Kinney1

1 Prothena Biosciences Inc, South San Francisco, California, USA. 2 Campbell Family Institute for Cancer Research, Ontario Cancer Institute/University Health Network, Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada. 3 Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada.

jeffrey.higaki@prothena.com

INTRODUCTION: We recently characterized conformation-specific monoclonal antibodies against amyloidogenic forms of TTR (mis-TTR mAbs) in vitro and demonstrated binding selectivity, including patient tissue.1 We showed that these antibodies can induce phagocytic clearance of aggregated TTR. Herein we have further characterized these mis-TTR mAbs using high-resolution microscopic techniques to directly evaluate their interactions with non-native conformations of TTR. In addition, we have assessed mis-TTR labeling by immunohistochemistry (IHC) in various diseased tissue with confirmed ATTR amyloidosis.

MATERIAL & METHODS: Antibodies were generated as previously described.1 Immunogold transmission electron microscopy (TEM) and atomic force microscopy (AFM) techniques were used to generate images of the interaction of mis-TTR mAbs with aggregated and fibrillar forms of the protein. Mis-TTR mAbs were also evaluated immunohistochemically to determine their reactivity to TTR amyloid deposits in heart, peripheral nerve, and gastrointestinal (GI) tract samples obtained from patients with diagnoses of ATTR amyloidosis.

RESULTS: We previously showed that conformation-specific mAbs induced antibody-dependent uptake of non-native and aggregated forms of TTR by THP-1 cells. In the present study, AFM and immunogold-labeled TEM images revealed a direct interaction between these mAbs and both fibrillar and aggregated forms of recombinant TTR. IHC with the mis-TTR antibodies and light microscopy revealed specific labeling of pericellular cardiac TTR deposits (TTR amyloid and pre-amyloid deposits), deposits in the endoneurium of peripheral nerves, and diffuse labeling of the muscularis (externa and mucosae), submucosal nerve plexus, and some vasculature of the GI tract. Normal control tissue and tissue with light chain (AL) amyloidosis showed no immunoreactivity. Further, the mis-TTR mAbs did not recognize native tetrameric TTR in human liver tissue.

DISCUSSION & CONCLUSIONS: Conformation-specific TTR mAbs immunoreact in vitro with an amyloidogenic epitope present only on non-native conformations of TTR and not on native tetrameric forms of the protein. mis-TTR mAbs induce phagocytic uptake of non-native and aggregated forms of TTR in vitro, and they specifically recognize TTR amyloid deposits in disease-confirmed heart, nerve, and GI tract tissue. These findings suggest that mis-TTR mAbs may prove useful in preventing deposition and/or enhancing clearance of TTR amyloid in patients with diagnoses of ATTR amyloidosis regardless of the specific organ(s) involved while sparing the function of the normal tetrameric form of the protein.

AL amyloidosis cell model expressing patient-derived immunoglobulin light and heavy chains

ES Klimtchuk, TB Prokaeva, BH Spencer, LH Connors

Amyloidosis Center, Boston University School of Medicine, Boston, Massachusetts, USA.

klimtchu@bu.edu

INTRODUCTION: In AL amyloidosis, monoclonal immunoglobulin (Ig) light chains (LC) form fibrillar deposits in various organs and tissues. The circulating monoclonal protein is detected by serum immunofixation electrophoresis as free LC or intact Ig, the latter most frequently of the IgG type. IgG is a heterotetrameric protein assembled from two identical LC and two identical heavy chain (HC) polypeptides connected by disulfide bonds. The proper folding and assembly of an intact Ig molecule is dependent on a variety of factors including protein sequence, expression levels, and chaperone interactions. Although amyloid fibrils are usually formed from monoclonal LCs and their fragments, several reports have shown amyloid deposits composed of HCs (1, 2). The aim of this work was to create an AL patient-derived monoclonal IgG cell model system that replicated the in vivo situation and could be used in the study of AL amyloidosis.

MATERIALS & METHODS: Bone marrow samples from 2 AL patients with circulating monoclonal intact IgG were used to clone IGV, IGJ, IGD, and IGC genes for LC and HC. Based on the sequences obtained, primers for LC and HC variable domains were designed and used for qPCR of bone marrow-derived mRNA to measure LC and HC gene expression. Recombinant, patient-specific LC and HC were cloned. Plasmacytoma Sp2/0 cells were nucleofected with AL LC/pMAZ-IgL and AL HC/pMAZ-IgH expression vectors (3). IgG proteins were expressed and purified from cell culture supernatants using Protein A-agarose resin. RNA was prepared from cell cultures, cDNA was synthesized, and LC and HC gene expression was measured by qPCR.

RESULTS: Genes used in rearrangement of LC and HC in 2 patient samples were identified as follows:

<table>
<thead>
<tr>
<th>Sample</th>
<th>LC gene</th>
<th>HC gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL-08071</td>
<td>IGKV1-39<em>01/IGKJ2</em>01/IGKC*01</td>
<td>IGHV1-69<em>02/IGHD5-24</em>01/IGHJ4<em>02/IGHG1</em>03</td>
</tr>
<tr>
<td>AL-05126</td>
<td>IGLV6-57<em>01/IGLJ3</em>01/IGLC3*04</td>
<td>IGHV3-30<em>03/IGHD3-3</em>02/IGHJ6<em>02/IGHG1</em>03</td>
</tr>
</tbody>
</table>

In both cases, HC proteins were found to be of IgG1 isotype. LC to HC gene expression ratios, found in bone marrow-purified mRNA samples of AL patients, varied from 1.7 to 4.3. The DNA fragments encoding LC and HC were inserted into mammalian vectors designed for human LC and human γ1 expression. A mouse plasmacytoma cell line Sp2/0, which does not express or secrete Ig, was chosen as a disease-relevant cell type for creation of the AL model. Stable cell lines were selected after co-transfection of recombinant LC and HC genes with patient-derived sequences; LC to HC gene expression ratios in selected cell lines reflected those measured in AL. Patient-specific LC and HC were expressed, assembled into IgG molecules, and secreted as confirmed by SDS-PAGE and immunoblot analyses.

DISCUSSION & CONCLUSIONS: We have created a cell model in which AL patient-specific monoclonal LC and HC proteins are expressed, assembled, and secreted as intact IgG1 proteins. This cell-based system is novel and competent, and can be used to represent the wide variety of LC and HC sequences featured in AL amyloidosis. The model has been designed to reflect monoclonal protein expression and secretion by patient plasma cells, and can be used for functional and mechanistic studies of AL amyloid disease.


This work was supported by the Wildflower Foundation, by the Gruss Foundation, and by the Amyloid Fund at Boston University.
**PA12**

**Targeting the toxic oligomers of amyloidogenic proteins by self-assembled cyclic D,L-α-peptides: Potential application for Alzheimer’s and Parkinson’s diseases**

S Rahimipour, M Chemerovski-Glikman, M Richman

1 Department of Chemistry, Bar-Ilan University, Ramat-Gan 5290002, Israel

rahimis@biu.ac.il

**INTRODUCTION:**

Protein misfolding and aggregation is the fundamental cause of more than 20 amyloidogenic diseases affecting either the central nervous system or a variety of peripheral tissues. Although peptides and proteins of various sequences can self-assemble into toxic amyloid structures, they share common three-dimensional features that may promote their cross-reaction. Given the significant structural and biochemical similarities between amyloids and the architecture of self-assembled cyclic D,L-α-peptides, we hypothesized that the latter may bind and stabilize the nontoxic forms of different amyloids, thereby preventing their aggregation into toxic forms.

**MATERIAL & METHODS:**

Peptides were synthesized using solid-phase peptide synthesis, employing the common Fmoc strategy. α-Synuclein (α-syn) and singly mutated [V66C]-α-syn was produced according to Wang et al [1]. [V66C]-α-syn was labeled with bromobimane and purified as described [2]. Thioflavin T (ThT) aggregation assay, kinetic assay for α-syn aggregation and disaggregation assay were performed as previously described [3, 4]. For HSQC NMR studies, 15N- and 15N/13C-labeled α-syn were expressed using M9 minimal medium containing 15NH4Cl in the absence or presence of 13C6-D-glucose. For sample preparation, a mixture of isotopically labeled α-syn (40 μM) and CP-2 (40 μM) was prepared in PB (50 mM, pH 7.4) and 7% D2O. An identical sample was prepared without CP-2 as a control. Samples were then aged with constant shaking (1050 rpm) in a NMR test tube for 3–6 days at 37 ºC and analyzed by NMR once every 24 h. Cell toxicity experiments were carried out on normal and α-syn-transfected SH-SY5Y neuroblastoma cell lines, using the MTT assay [4]. The internalization of CP-2 into SH-SYSY cells and its intracellular localization were studied using the NBD-labeled CP-2 derivative (CP-3) and the LysoTracker® reagent. Cells were grown and treated with CP-3 (2 μM) for 4 h, washed with fresh DMEM containing 10% FBS medium and stained with LysoTracker® and Hoechst for 30 min. Cell imaging was then performed on a confocal microscope.

**RESULTS and DISCUSSION**

By screening an unbiased library of six-residue cyclic D,L-α-peptides and optimizing the activity of a lead peptide, we previously found one cyclic D,L-α-peptide (CP-2) that interacts strongly with Alzheimer’s disease associated Amyloid beta (Aβ) and inhibits its aggregation and toxicity [4]. Using further Thioflavin T assays, electron microscopy, and circular dichroism spectroscopy, we have found that CP-2 could also effectively cross-interact with Parkinson’s disease associated α-synuclein (α-syn), prevent its aggregation, and remodels its fibrils to non-toxic amorphous species, through an “off pathway” mechanism. NMR and FRET studies show that CP-2 interacts with the N-terminal and the non-αB component region of α-syn, which are responsible for α-syn’s membrane interactions and self-assembly, and so changes its conformation. Dot-blot assays using oligomer-specific antibody (A11) and cell survival experiments suggest that CP-2 reduces the amount of toxic α-syn oligomers and protects PC-12 and SH-SYSY cells from α-syn induced toxicity. We found that CP-2 utilizes the same mechanism used by amyloids i.e. endocytosis to internalize cells. Moreover, upon its internalization CP-2 co-localize with intracellular α-syn and reduces its accumulation and toxicity in neuronal cells overexpressing α-syn. Our studies suggest CP-2 acts as a conformational inhibitor of different amyloids due to its immense structural and functional similarities to the amyloids and, that targeting the common structural conformation of amyloids may be a promising approach for developing new therapeutics for amyloidogenic diseases.

**REFERENCES**

Conformation dependent seeding of fibril formation from SAA1.1 protein in cell culture

K Meinhardt¹, S Claus¹, C Loos¹, F Liberta¹, M Fändrich¹

¹Institute of Protein Biochemistry, Ulm University, Ulm, Germany.
cornelia.loos@uni-ulm.de

INTRODUCTION: Amyloid A (AA) amyloidosis can be studied in mouse and cell culture models and amyloid deposition can in both systems be dramatically accelerated by the addition of amyloid enhancing factor (AEF). It was suggested that AA fibrils might be the molecular basis of AEF activity [1]. Here we show that in vitro generated fibrils of murine SAA1.1 alone can promote amyloid deposition in cell culture in an AEF like fashion. However, the seeding activity was strongly dependent on the conformation of the fibrils.

MATERIAL & METHODS: J774A.1 cell culture, cell viability assays, Congo red green birefringence, Thioflavin T and Congo red binding assays, transmission electron microscopy (TEM), persistence length measurements, X-ray diffraction measurement, infrared spectroscopy.

RESULTS: The tested SAA1.1 fibrils were either extracted from a cell culture model or grown from pure SAA1.1 protein in vitro at pH 3 or pH 8. Typical amyloid characteristics like cross-β structure, binding of Congo red and Thioflavin T were confirmed for all three fibril preparations.

The incubation of J774A.1 cells with fibrils from cell culture or grown in pH 8 results in a drastic increase of the amyloid deposits. However, fibrils grown in pH 3 barely seed for fibril formation.

Additionally, the cell culture fibrils are more toxic than the fibrils, which were generated in buffer. As shown by MTT assay, cell culture fibrils strongly reduced the cellular viability, whereas the in vitro generated fibrils scarcely affected the viability of the cells.

The analysis of the kinetics of the fibril formation reveal that cell culture and pH 8 fibrils strongly reduce the lag phase of fibril formation, while pH 3 fibrils present only very modest seeding activity.

The morphological analysis with TEM shows that cell culture fibrils, as well as fibrils grown in pH 8 buffer, are long and straight, and thus similar to ex vivo fibrils from AA amyloidotic tissues. Fibrils grown at pH 3, however, display a curvilinear phenotype. These bending characteristics were quantified through persistence length measurements.

DISCUSSION & CONCLUSIONS: Our data support the view that the seeding active component of the AEF is the AA fibril itself [1], as the morphology and the protein conformation evidently influence the toxicity and seeding efficacy of the fibril preparation.

Human Transthyretin Modulates Fibrillation and Contributes to Biofilm Inhibition in E. coli

Neha Jain, Brennan McMichael, Xinyi Li, Joel N Buxbaum and Matthew R Chapman

Amyloidogenesis is under intense scrutiny due to its intimate association with neurodegenerative disorders like Parkinson’s and Alzheimer’s diseases. Recent years have witnessed the emergence of a new class of amyloids designated as ‘functional amyloids’ where the amyloid-fold is not deleterious, but instead is harnessed to perform diverse functions. One of the best-studied functional amyloids is curli fibers produced by many Gram-negative bacteria. The major curli subunit, CsgA (curli specific gene A), is assembled into β-sheet rich amyloid fibres on the cell surface and contributes to biofilm formation.

The present study focuses on the modulation of curli assembly by a human chaperone-like protein called transthyretin (TTR). Previously, human transthyretin was shown to inhibit Aβ (amyloid β) aggregation and thereby reduction in its pathogenic effects. Here, we report that interactions between CsgA and TTR lead to amyloid inhibition both in vitro and in vivo. In vitro, freshly purified CsgA forms Thioflavin-T and Congo-red positive amyloid fibrils. Co-incubation of CsgA with human tetrameric TTR, or its engineered monomeric form (F87M/L110M) [MTTR], resulted in inhibition of CsgA fibrillogenesis. When mixed with TTR, CsgA was unable to adopt the β-sheet conformation and remained soluble and unaggregated. Monomeric TTR was even more efficient than tetrameric TTR at discouraging CsgA amyloid formation. Monomeric TTR was also capable of inhibiting seeded aggregation of CsgA, suggesting that it can act on both primary and secondary nucleation. Interestingly, TTR was also shown to be an effective anti-biofilm protein. When TTR was added to E. coli cells grown under biofilm-inducing conditions, there was extensive biofilm inhibition with no detectable effect on bacterial growth. Our results have identified a new biofilm inhibitor and suggest potential therapeutic interventions that forgo traditional antibiotics.
Nanoscale biophysics of amyloids: Mechanism and structural organization

S. Mukhopadhyay

Centre for Protein Science, Design and Engineering, Department of Biological Sciences and Department of Chemical Sciences, Indian Institute of Science Education and Research (IISER), Mohali, India.
mukhopadhyay@iisermohali.ac.in

Amyloids are ordered protein aggregates that are linked to a variety of devastating human diseases such as Alzheimer’s, Parkinson’s, prion diseases and systemic amyloidosis. The transition from a normal functional protein to an altered (misfolded) form involves a profound conformational change that triggers the abnormal protein aggregation resulting in a wide variety of nanostructures including amyloid oligomers, pores and fibrils. My laboratory utilizes a diverse range of methodologies to unravel the key molecular events that are crucial in amyloid formation from a number of proteins including β₂-microglobulin, α-synuclein and the prion protein. Using Raman spectroscopy in combination with atomic force microscopy (AFM), we have been able to delineate the key structural transitions during amyloid formation. The AFM images revealed a progressive morphological transition from spherical oligomers to nanoscopic annular pores (Fig. 1a), whereas, the Raman data indicated the protein structural changes during amyloid assembly and pore formation. Recently, we have used the combination of AFM and Raman to monitor the structural transition of human prion protein into protease-resistant amyloid oligomers that assemble into ordered fibrils (Fig. 1b). Additionally, we have adapted a super-resolution nanophotonic technology that allows us to optically image individual amyloids at the nanoscopic spatial resolution. We have utilized near-field scanning fluorescence microscopy to optically map the amyloid fibrils of β₂-microglobulin far beyond the diffraction-limit. Interrogation of individual fibrils by simultaneously monitoring both nanoscale topography (Fig. 1c) and fluorescence brightness (Fig. 1d) revealed heterogeneous packing of the cross-β architecture within amyloids. Our results provide structural mechanistic underpinnings of diverse amyloid polymorphs that underlie the strain phenomenon in amyloid biology.


Fig. 1: AFM images of (a) an amyloid pore and (b) human prion protein fibrils. Correlated nanoscale topography (c) and fluorescence (b) images of amyloid fibrils under near-field scanning optical microscopy.
Lack of lactadherin, the medin precursor protein, reduces cerebral β-amyloidosis in a mouse model of Alzheimer’s Disease

K Degenhardt1,2, D del Turco3, HA Davies4, U Obermueller1,2, J Madine4, T Deller3, M Jucker1,2, JJ Neher1,2

1Department of Cellular Neurology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany. 2German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany. 3Institute of Clinical Neuroanatomy, Neuroscience Center, J.W. Goethe University, Frankfurt/Main, Germany. 4Institute of Integrative Biology, University of Liverpool, Liverpool, UK.

INTRODUCTION: The amyloidogenic peptide medin is a 50 amino acid-long fragment of the protein lactadherin (also known as Milk fat globule EGF-like factor 8, MFG-E8). Medin is known to form aortic medial amyloid (AMed) localized in the arteries of the upper part of the body of virtually anybody over 60 years of age1. Recent data indicate that medin may co-aggregate with other amyloids, such as serum amyloid A 2. However, whether medin may influence the aggregation of amyloid-β, the amyloid deposited in insoluble plaques in Alzheimer’s disease, remains unknown.

MATERIAL & METHODS: We crossed a mouse model of cerebral β-amyloidosis, APPPS1 mice3, with Mfge8 knockout animals4. We then analysed plaque load as well as MFG-E8 immunoreactivity by immunohistochemistry and electron microscopy. Furthermore, we performed amyloid extractions and determined whether medin may co-purify with amyloid-β.

RESULTS: Here we find that knockout of the medin precursor protein, MFG-E8, leads to reduced plaque load in the APPPS1 mouse model of Alzheimer’s Disease. Strikingly, in APPPS1 x Mfge8+/+ mice, immunostaining for MFG-E8 showed very strong co-localization with amyloid plaques and electron microscopy revealed that this MFG-E8 immunoreactivity localized to amyloid fibrils, with staining being absent in APPPS1 x Mfge8-/- animals. Furthermore, the levels of MFG-E8 increased both with age and cerebral β-amyloidosis. Importantly, amyloid extractions both from diseased mouse and human brain revealed enrichment of a medin-immunoreactive peptide together with amyloid-β.

DISCUSSION & CONCLUSIONS: Our work may indicate that MFG-E8 and/or medin promote cerebral β-amyloidosis through a direct interaction with amyloid-β. Thus, future work will investigate whether medin aggregates can cross-seed amyloid-β and may thereby promote Alzheimer’s disease.

REFERENCES:

INTRODUCTION:
Amyloid formation of the plasma protein transthyretin (TTR) has been linked to familial amyloid polyneuropathy and senile systemic amyloidosis. Binding of ligands within its natural hormone binding site can stabilize the tetrameric structure and impair amyloid formation. We have recently shown that the flavonoid luteolin stabilizes TTR in human plasma with a very high selectivity. Luteolin, however, is inactivated \textit{in vivo} via glucuronidation for which the preferred site is the hydroxy group at position 7 on its aromatic A-ring.

MATERIAL & METHODS:
We have evaluated the properties of two luteolin variants in which the 7-hydroxy group has been exchanged for a chlorine (7-Cl-Lut) or a methoxy group (7-MeO-Lut).

RESULTS:
Using an \textit{in vitro} model, based on human liver microsomes, we verified that these modifications increase the persistence of the drug. Crystal structure determinations show that 7-Cl-Lut binds similarly to luteolin. The larger MeO substituent cannot be accommodated within the same space as the chlorine or hydroxy group and as a result 7-MeO-Lut binds in the opposite direction with the methoxy group in position 7 facing the solvent. Both 7-Cl-Lut and 7-MeO-Lut qualify as high-affinity binders, but in contrast to luteolin, they display a high non-specific binding to other plasma components. The binding of the two conformations and the key-interactions to TTR are discussed in detail.

DISCUSSION & CONCLUSIONS:
Taken together these results show a proof-of-concept that the persistence of luteolin towards enzymatic modification can be increased. We reveal two alternative high-affinity binding modes of luteolin to TTR and that modification in position 7 is restricted only to small substituents if the original orientation of luteolin should be preserved. In addition, the present work provides a general and convenient method to evaluate the efficacy of TTR-stabilizing drugs under conditions similar to an \textit{in vivo} environment.
Water soluble gold nanoparticles: a new tool in amyloid research

PJ Silva¹, U Cendrowska¹, N Ait-Bouziad², H Lashuel², F Stellacci¹

¹ Institute of Materials, Ecole Polytechnique Fédérale de Lausanne, EPFL-STI-IMX-SuNMIL, Lausanne CH-1015, Switzerland.
² Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne, EPFL-SV-BMI-LMNN, Lausanne CH-1015, Switzerland.
paulo.jacob@epfl.ch

INTRODUCTION: A variety of proteins can self-assemble into amyloid-like fibrils. Such conversion can be associated with several human diseases, including neurodegenerative disorders. Unravelling the molecular basis of diseases caused by protein misfolding is a complex task and as studies progress new tools and methods are required. Nanoparticles and their potential applications can meet some of the demands in this field. We have found that amphiphilic gold nanoparticles protected by a mixture of sulfonated (11-mercapto-1-undecanesulfonate) and hydrophobic (1-octanethiol) molecules can discriminatively adsorb onto surface features of amyloid fibers made of Aβ₁⁻⁴₀ and α-synuclein in vitro, and that hydrophobicity determines adsorption onto fibers made of Tau-441 protein. We also show that these nanoparticles adsorb onto small oligomers and possibly secondary nuclei of Aβ₁⁻⁴₂ before occupying the edges of amyloid-like fibers.

MATERIAL & METHODS: Monolayer protected gold nanoparticles were synthesized with a variety of chemical functionalities on their surfaces, at a length scale commensurate with the features found in amyloid-like fibers and their building blocks. The nanoparticles were incubated either with mature amyloid-like fibers or monomers of Aβ₁⁻⁴₀, Aβ₁⁻⁴₂, α-synuclein and Tau-441 in various conditions. Images of fibers, oligomers and nanoparticles were taken by cryogenic electron microscopy (cryo-EM) among other microscopy techniques.

RESULTS: Our group has established how different types of water soluble gold nanoparticles, ranging from 1 to 6 nm in diameter interact with different types of amyloid-like fibers and their precursors. Given a fiber that adopts a twisted ribbon morphology, these amphiphilic gold nanoparticles adsorb onto the edges of these structures, leaving other interfaces uncovered. This generates a novel supra-molecular assembly that directly interfaces an engineered nanomaterial with a biological structure, without the use antibodies, and can be a useful marker for electron microscopy imaging of proteins in the amyloid state. Experiments and calculations demonstrated the importance of nanoparticle size and ligand-shell composition: small sulfonated amphiphilic nanoparticles mark amyloid fibers and highlight their three-dimensional structure under cryo-EM.

DISCUSSION & CONCLUSIONS: We present a new class of materials that can become a tool in amyloid research and possibly help cross-instrumental spectroscopic and imaging techniques for molecular structure determination, track amyloid fibers and oligomers in vivo and easily visualize secondary nucleation and the effect of drugs designed to block this process.

REFERENCES:

Fig. 1: Cryo-EM image of alpha synuclein amyloid-like fibers discriminatively decorated with water-soluble gold nanoparticles.
ABT-199 (Venetoclax) for relapsed refractory systemic AL amyloidosis (AL): pre-clinical studies and a role for sensitivity testing of CD138-selected marrow plasma cells

P Zhou1, X Ma1, SW Wong1, M Warner1, J Cowan2, R L Comenzo1

1The John C Davis Myeloma and Amyloid Program, Tufts Medical Center, Boston, MA, USA
2Department of Pathology and Laboratory Medicine, Tufts Medical Center, Boston, MA, USA

pzhou@tuftsmedicalcenter.org

Despite advances in therapy for AL, three major unmet medical needs remain: earlier diagnosis of cardiac involvement, reversal of amyloid-related organ damage, and relapsed refractory plasma cell disease. Only a quarter of patients at diagnosis are eligible for autologous stem cell transplant (SCT) with risk-adapted melphalan, of whom 60% achieve hematologic CR with post-SCT bortezomib, while less than half of transplant ineligible patients achieve > VGPR with bortezomib-based initial therapy. Furthermore, there are no standard therapies for reversal of organ damage or relapsed disease. New and better anti-amyloid and anti-plasma cell therapies are needed.

Bcl-2 inhibition represents a promising therapeutic approach to B-cell malignancies. The family of Bcl-2 proteins regulates intrinsic apoptosis by affecting mitochondrial membrane depolarization. Bcl-2 proteins include anti-apoptotic proteins Bcl-2, Bcl-xl and Mcl-1 that function by sequestering pro-apoptotic activator proteins, particularly the BH3-only protein Bim. Clonal plasma cells are primarily dependent on the anti-apoptotic activity of Mcl-1 but can be co-dependent on Bcl-2 and Bcl-xl also. ABT-199 (Venetoclax) is the first-in-class orally bioavailable Bcl-2-selective BH3 mimetic and acts by displacing Bim from Bcl-2, enabling Bim to bind to and activate the effector proteins Bax and Bak, triggering mitochondrial depolarization and intrinsic apoptosis. ABT-199 has been shown to be active and well tolerated in patients with chronic lymphocytic leukemia. In vitro, ABT-199 has shown activity in multiple myeloma cell lines harboring t(11;14), in part because there may be greater Bcl-2 co-dependence in such clones.

To study ABT-199 activity in AL, we tested ABT-199 in vitro at 100nM in bioluminescent caspase 3/7 activity assays with CD138-selected cells from 16 patients with relapsed AL. We used KMS-12-PE cells as positive and NCI-H929 cells as negative controls with each assay. KMS-12-PE cells contain t (11;14). Our read-out was the ratio of caspase 3/7 activity in ABT-199 treated to untreated samples. In Figure 1A below, the results (mean+/− SD) for all patient samples (n=16), sensitive patient samples (S, n=8), resistant patient samples (R, n=8), and positive and negative controls are shown with comparisons to negative controls and R samples by unpaired t-test (*P < 0.05). CD138+ cells from half of the AL patients were as sensitive to ABT-199 as KMS-12-PE cells. Furthermore, with fluorescent in situ hybridization (FISH) performed on CD138-selected cells from the same marrows, in Figure 1B we show that patient cells containing t(11;14) were significantly more sensitive (*) to ABT-199 than cells with other findings by FISH.

In conclusion, these pre-clinical data provide the rationale for a phase I trial of ABT-199 and dexamethasone in patients with relapsed refractory AL whose CD138-selected clonal plasma cells are sensitive to ABT-199 in vitro.

Figure 1

Intrinsic cellular stress underlies exquisite proteasome inhibitor susceptibility in amyloidogenic plasma cells

L Oliva, U Orfanelli, M Resnati, A Raimondi, A Orsi, E Milan, G Palladini, P Milani, F Cerruti, P Cascio, S Casarini, P Rognoni, T Touvier, M Marcatti, F Ciceri, G Merlini, S Cenci

1 San Raffaele Scientific Institute, Division of Genetics and Cell Biology, Unit of Age Related Diseases, Milano, Italy. 2 San Raffaele Scientific Institute, Imaging Research Center, Milano, Italy. 3 Amyloidosis Research and Treatment Center, Fondazione IRCCS Policlinico San Matteo, and Department of Molecular Medicine, University of Pavia, Pavia, Italy. 4 Department of Veterinary Sciences, University of Torino, Italy. 5 San Raffaele Scientific Institute, Division of Genetics and Cell Biology, Unit of Biology of Myelin, Milano, Italy. 6 San Raffaele Scientific Institute, Department of Oncohematology, Hematology and Bone Marrow Transplantation Unit, Milano, Italy. 7 Università Vita-Salute San Raffaele, Milano, Italy.

INTRODUCTION: Systemic light chain (AL) amyloidosis is a plasma cell (PC) dyscrasia caused by the clonal production of an unstable immunoglobulin light chain (LC), which affects organ function systemically. While pathogenic LCs have been characterized biochemically, little is known about the biology of amyloidogenic PCs. Encouraged by the unique response rates of AL amyloidosis patients to the first-in-class proteasome inhibitor (PI) bortezomib, we purified and investigated patient-derived AL PCs, in comparison with primary multiple myeloma (MM) PCs, the prototypical PI-responsive cells.

MATERIAL & METHODS: By multiple immuno-magnetic sorting, we purified and characterized, for the first time, PCs from AL amyloidosis patients, in comparison with primary PCs from multiple myeloma (MM) and monoclonal gammopathy undetermined significance (MGUS). Integrating multiple approaches (enzymatic and apoptotic assays, immunofluorescence, protein and transcript biochemistry, flow cytometry, electron microscopy, and cytochemistry), we assessed PI sensitivity, proteasome activity and workload, endoplasmic reticulum (ER) and mitochondrial homeostasis, and activation of cellular and organelle-specific stress response pathways. Moreover, to establish the causal role of amyloidogenic LC production in conferring stress and PI sensitivity, we lentivirally engineered stable PC lines that can be induced to express patient-derived amyloidogenic and non-amyloidogenic LC.

RESULTS: Functional, biochemical and morphological characterization revealed an unprecedented intrinsic sensitivity of AL amyloidogenic PCs to PIs, even higher than that of MM PCs, associated with distinctive organelar features and expression patterns indicative of cellular stress. These consisted in expanded endoplasmic reticulum (ER), perinuclear mitochondria, and higher abundance of stress-related transcripts, and were consistent with reduced autophagic control of organelle homeostasis, in view of the critical role played by autophagy in normal and malignant PCs. In engineered PC lines, induction of amyloidogenic LC expression was sufficient to confer PI sensitivity, establishing that the stress experienced by AL PCs is a direct and cell-intrinsic consequence of amyloidogenic LC production.

DISCUSSION & CONCLUSIONS: Our study discloses amyloidogenic LC production as a cellular stressor, and identifies stress-responsive pathways as potential therapeutic targets. Moreover, we contribute a cellular disease model to dissect the biology of AL PCs.

REFERENCES:
Antiamyloidogenic and proamyloidogenic chaperone effects of C-reactive protein and serum amyloid P component

D Ozawa1, R Nomura1, PP Mangione2, K Hasegawa1, T Okoshi1, R Porcari2, V Bellotti2, H Naiki1

1 Department of Pathological Sciences, Faculty of Medical Sciences, University of Fukui, Fukui, Japan. 
2 Wolfson Drug Discovery Unit, Centre for Amyloidosis and Acute Phase Proteins, Division of Medicine, University College London, London, UK.

ozawa@u-fukui.ac.jp

INTRODUCTION: C-reactive protein (CRP) and serum amyloid P component (SAP), two major classical pentraxins in humans, are soluble pattern recognition molecules that regulate the innate immune system. They have a unique pentameric structure and bind to their ligands calcium-dependently with their B faces. Pentameric CRP binds calcium-independently to various proteins, including amyloid β (Aβ), at acidic pH in vitro1. It is hypothesized that pentameric CRP protects against toxic conditions caused by protein misfolding and aggregation in acidic inflammatory environments, but the chaperone activity of CRP remain poorly understood. SAP is present universally in all extracellular amyloid deposits2. Its primary role in amyloidogenesis is thought to enhance the formation and deposition of amyloid fibrils by binding to the surface of amyloid fibrils calcium-dependently with the B face. On the other hand, SAP inhibits the amyloid fibril formation of Aβ3 and enhances the refolding yield of denatured lactate dehydrogenase in vitro4. No convincing data or models have been published thus far to explain the discrepancy between the pro- and anti-amyloidogenic activities of SAP. In this study, we investigated the effects of CRP and SAP on amyloid fibril formation and amorphous protein aggregation in vitro.

MATERIAL & METHODS: Human CRP and SAP were kind gifts from Professor Mark B. Pepys, UCL. Amyloid fibril formation was monitored by thioflavin T assay and electron microscopy. Amorphous protein aggregation was monitored by turbidity assay. Protein-protein interaction was assessed by enzyme-linked immunosorbent assay and western blotting analysis.

RESULTS: CRP and SAP dose-dependently and substoichiometrically inhibited both Aβ(1-40) and D76N β2-microglobulin (β2-m) fibril formation in a Ca2+-independent manner. CRP and SAP interacted with fresh and preaggregated Aβ(1-40) and D76N β2-m on the fibril-forming pathway. Interestingly, in the presence of Ca2+, SAP first inhibited, then significantly accelerated D76N β2-m fibril formation. Electron microscopically, the surface of the D76N β2-m fibril was coated with pentameric SAP. These data suggest that SAP first exhibits antiamyloidogenic activity possibly via A face, followed by proamyloidogenic activity via B face. Finally, SAP inhibited the heat-induced amorphous aggregation of human glutathione S-transferase.

DISCUSSION & CONCLUSIONS: Amyloid fibrils and amorphous aggregates are two types of aberrant aggregates associated with protein misfolding diseases. The present study showed that CRP and SAP inhibit amyloid fibril formation in vitro. We also showed that SAP has chaperone activity that inhibits not only amyloid fibril formation but also amorphous protein aggregation in vitro. We obtained new insight into the chaperone activity of pentraxins, proposing that 1) classical pentraxins (CRP, SAP) may be a member of extracellular chaperones, and 2) the pro- and anti-amyloidogenic activities of SAP are not mutually exclusive, but reflect two sides of the same coin.

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**PROTEINS ACCOMPANYING AL, ATTR AND ALECT2 IN HEART AND RENAL AMYLOID PLAQUES**

TV Kourelis¹, S Dasari², Theis JD³, JA Vrana², PJ Kurtin³, AD Dispenzieri¹

¹Division of Hematology, ²Department of Health Sciences Research, ³Department of Laboratory Medicine and Pathology Mayo Clinic, MN, email: kourelis.taxiarchis@mayo.edu

**INTRODUCTION:** Several non-amyloidogenic proteins are co-deposited in amyloid plaques along with the amyloid forming protein and are thought to play an important role in amyloidogenesis and organ toxicity. Herein, using a shotgun proteomics approach, we describe the non-amyloid protein distribution and abundance in amyloid deposits from cardiac and renal tissue specimens of patients with light chain (AL), transthyretin (TTR) and ALECT2 amyloidosis.

**MATERIAL & METHODS:** Laser microdissection and mass spectrometry (LMD/MS) were performed in 83 Congo red positive specimens as previously described [1]. At least 3 separate LMDs/specimen were performed. The non-amyloid proteins were considered only if they were identified with >95% confidence and if an average of ≥ 5 spectral counts was present across all LMDs. For quantitative analyses, the total number of spectra matched in a sample was used as a normalization factor. Thirty-five renal (29 AL, 6 ALECT2) and 48 heart (30 AL, 18 TTR) samples were included in this study.

**RESULTS:** A median of 11 non-amyloid proteins/sample were present. The distribution of these is shown in the figure. When considering their abundance in AL samples, APOE and heparan sulphate were more abundant in renal samples and myosin more abundant in cardiac samples (p<0.01). In cardiac samples, SAP was more abundant in TTR patients (p<0.0001). In renal samples vitronectin was more abundant in ALECT2 patients than AL patients (p=0.001). In cardiac AL samples, the non-amyloid protein distribution or abundance did not differ in patients experiencing an early death (within 6 months from diagnosis). Patients with NTproBNP levels above the median had higher collagen VI levels (p<0.01). In renal AL samples, non-amyloid protein distribution or abundance did not differ in patients with 24 hr. urine protein levels above and below the median.

**DISCUSSION & CONCLUSIONS:** Amyloid plaques have a rich repertoire of non-amyloid proteins, some of which have been shown to promote/inhibit amyloidogenesis (e.g. clusterin, collagen, SAP, heparan). Chaperone distribution and abundance differs between different tissue and amyloid types. Treatments targeting some these are already in development (e.g. anti-SAP antibodies in AL, eprodise for heparan in AA). These analyses are underpowered but are hypothesis generating and suggest that a proteomics approach in a larger number of samples and using appropriate tissue controls is required to explore the impact of the amyloid nano-environment on disease biology and patient outcomes.


**Figure.** Non-amyloid protein distribution in renal and heart samples*

*Only proteins present in at least 10% of either heart or renal samples are shown, **p<0.001 across same tissue type
IMMUNOGLOBULIN VARIABLE REGION GENE USAGE AND HEAVY CHAIN SEEDING IN LOCALIZED AND SYSTEMIC LIGHT CHAIN AMYLOIDOSIS

TV Kourelis1, S Dasari2, JA Vrana2, JD Theis3, PJ Kurtin3, AD Dispenzieri1

1 Division of Hematology, 2Department of Health Sciences Research, 3Department of Laboratory Medicine and Pathology Mayo Clinic, MN, email: kourelis.taxiarchis@mayo.edu

INTRODUCTION: Prior studies have hypothesized that organ tropism in AL amyloidosis is a function of immunoglobulin variable region germ line (IgV) gene usage. We have refined our proteomics methodology and are able to accurately identify IgV gene usage in clinical tissue specimens used for amyloid subtyping.

MATERIAL & METHODS: Laser microdissection and mass spectrometry (LMD/MS) were performed in 722 Congo red positive specimens as previously described [1]. At least 3 separate LMDs/specimen were performed. IgV genes were considered only if they were identified with >95% confidence and if an average of ≥ 5 spectral counts was present across all LMDs. This method for identifying IgV genes was validated in an independent population (n=30) by comparing it to mRNA sequencing of bone marrow samples. In order to minimize the influence of serum contamination, only non IgG heavy chain isotypes were considered and only if they fulfilled the criteria mentioned above.

RESULTS: Of 722 patients, 90 had localized and 632 systemic AL. IgV gene was successfully identified in 88% of cases. LV6-57 was overrepresented and KV3-20 underrepresented in systemic AL (figure). LV6-57, LV3-01, LV2-14, LV1-44, KV1-33 and LV3-21 were identified in 51% of cases. Co-deposition of heavy chains was more common in localized AL (figure). When considering patients with cardiac involvement heavy chain deposition was less common compared to patients without cardiac involvement (p<0.001). In patients with renal involvement, LV6-57 was overrepresented (p<0.0001) and heavy chains less frequently co-deposited (p<0.01) compared to patients without renal involvement.

DISCUSSION & CONCLUSIONS: This is the largest cohort to date to describe IgV gene usage in AL. We demonstrate that IgV gene usage is restricted in AL and differs between systemic and localized disease. Our results suggest that IgV gene usage only partially (i.e. renal involvement) explains organ tropism in AL. Heavy chain deposition is less common in systemic disease and especially in patients with cardiac and renal involvement. The mechanism and significance underlying this observation is unclear but this provides novel insights into the pathophysiology of AL.


Figure. IgV gene usage heavy chain seeding in systemic and localized AL
**PRELIMINARY CHARACTERIZATION OF A NOVEL PEPTIDE-FC-FUSION CONSTRUCT FOR TARGETING AMYLOID DEPOSITS**

JS Foster¹, R Koul¹, A Williams¹, EB Martin¹, T Richey¹, A Stuckey², S Macy¹, SJ Kennel¹,², and JS Wall¹,²

¹ Department of Medicine, University of Tennessee Medical Center, Knoxville, USA. ² Department of Radiology, University of Tennessee Medical Center, Knoxville, USA.

INTRODUCTION: Fc-fusion constructs are synthetic biomolecules composed of an Fc immunoglobulin domain linked to another protein or peptide. The Fc domain endows two major benefits to the peptide of interest; principally, enhancement of the plasma half-life due to its interaction with the neonatal Fc-receptor, and the ability to engage and activate effector immune cells via interactions through membrane-bound Fc-receptors. Numerous Fc-fusion proteins have been approved by the US FDA for indications such as rheumatoid arthritis (etanercept: TNFR) and Cryopyrin-associated periodic syndromes (rilonacept: IL-1R). To generate a novel, potentially immunotherapeutic, reagent for targeting systemic amyloidosis we synthesized a murine Fc-fusion construct that incorporates the synthetic amyloidophilic peptide p5 with a murine IgG2a Fc (Fig. A). The murine Fc was used in this preliminary fusion to allow proof of principle studies in mouse models of amyloidosis.

MATERIALS & METHODS: The pFUSE-mIgG2A-Fc vector, expressing the CH2 and CH3 domains of the murine IgG2a heavy chain, was purchased from InvivoGen (San Diego, CA). The cDNA for peptide p5 with a five amino acid spacer was purchased from Integrated DNA Technologies (Coralville, IA). The p5 cDNA was cloned into the vector via In-Fusion cloning. The vector was transiently transfected into HEK293T/17 and CHOK1 cell lines cultured in serum free medium. Secreted Fcp5 was purified from the medium using a protein A-conjugated matrix. Binding of the purified Fcp5 with amyloid was demonstrated immunohistochemically and by using a pulldown assay. Reactivity with murine AA amyloid in vivo was assessed by microautoradiography using ¹²⁵I-Fcp5. Fcp5-mediated phagocytosis of amyloid extracts and synthetic fibrils was measured in vitro using the RAW264.7 murine macrophage cell line in a quantitative pHrodo-green fluorescence assay.

RESULTS: Fcp5 fusion was expressed in both HEK and CHO cell lines at ~1-5 µg/mL of culture medium. In the pulldown assay ¹²⁵I-Fcp5 bound Aβ(1-40), IAPP, and rVλ6Wil synthetic fibrils with >90% of radiolabeled material in the fibril pellet. Additionally, the ¹²⁵I-Fcp5 bound human AL amyloid extracts, albeit with less efficiency than the fibrils. The Fcp5 fusion specifically localized with amyloid deposits in formalin-fixed paraffin embedded tissue sections and was shown to bind human ATTR (Fig. B), ALλ, ALκ, Aβ, and canine AA, confirming that the multi-amyloid reactivity of the p5 peptide was preserved. In mice with AA amyloid ¹²⁵I-Fcp5 specifically bound the amyloid deposits in all organs and tissues as evidenced by microautoradiography. Finally, Fcp5 effectively mediated the phagocytosis of pHrodo green-labeled human amyloid extracts and synthetic fibrils in vitro.

DISCUSSION: Based on these positive preliminary data the Fcp5 fusion, or a similar constructs employing other amyloidophilic peptides, may provide a novel reagent for targeting an immunologically active biomolecule to amyloid to expedite clearance of the deposits in patients. These reagents may perform as effectively as current fibril-reactive antibodies but additionally could provide pan-amyloid reactivity.
A novel mass spectrometry-based assay for the diagnosis and typing of systemic amyloidosis

PJ Boersema¹, J Bijzet², CE Zimmerli¹, BP Hazenberg², P Picotti¹

¹ Institute of Biochemistry, EHT Zürich, Zürich, Switzerland. ² Department of Rheumatology & Clinical Immunology, University Medical Center Groningen, Groningen, The Netherlands.

b.p.c.hazenberg@umcg.nl

INTRODUCTION: The current diagnostic approach for amyloidosis is tedious and relies on several different biochemical techniques. Biomolecular mass spectrometry (MS) can be used in medical diagnosis to identify and quantify protein levels. Here we developed an MS-based method that assays in one analysis the major specific systemic amyloidosis proteins, their eventual mutations and several additional proteins that may function as generic indicators of systemic amyloidosis.

MATERIAL & METHODS: Fat tissue aspirates were processed for liquid chromatography (LC-)MS analysis. A selected reaction monitoring (SRM)-MS assay was developed targeting 13 major systemic amyloidosis proteins (including TTR, Ig-Lambda, Ig-Kappa and SAA) and often occurring mutations on these proteins (11 for TTR, 2 for FGA, 1 for Lys, 1 for ApoA1). Furthermore, 20 additional proteins, selected from shotgun proteomics measurements, were included in the assay to evaluate their diagnostic potential for generic systemic amyloidosis. All proteins and mutations were measured using 1 µg of a fat tissue sample during a single MS analysis with a 35 min LC gradient. Heavy isotope-labelled synthetic peptide analogues for all targeted peptides were spiked in the sample to validate detection and enhance quantification. Congo red-stained amyloid in fat smears was graded visually in a semi-quantitative way, ranging from 1+ (<1% of the inspected surface), 2+ (1-10%), 3+ (10-60%) to 4+ (>60%). Fifty-eight samples were used for the training set and 84 samples will be used for validation.

RESULTS: Fat tissue aspirates dissolved in Guanidine-HCl were directly processed for LC-MS (trypsin digestion). After untargeted (shotgun) proteomics analysis, 20 proteins were shortlisted as potential systemic amyloidosis signature proteins. Fifty-eight samples for the training set - consisting of control samples and patient samples with varying levels of ATTR, AA, AL-lambda and AL-kappa - were measured with the SRM-assay. In most cases the amyloidogenic protein was detected; >80% specificity and sensitivity for the whole group. Possibly present mutations could also be detected in the same assay. Correctness of the disease diagnosis increased with increasing amyloid grade (Table 1). ATTR and AA could be diagnosed correctly in almost all cases, AL-lambda and AL-kappa diagnosis had a lower sensitivity. Amyloid signature proteins could be used to diagnose the presence of amyloid fibrils in fat tissue aspirates from systemic amyloidosis patients regardless of type. Also the concentration of `signature' proteins generally increased with amyloid grade. For validation a set of 84 blinded samples will be used.

Table 1. Amyloid grade (1-4+) of fat tissue and correct typing of amyloid using SRM-MS in the training set.

<table>
<thead>
<tr>
<th>SRM-MS result</th>
<th>Amyloid grade</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td>Amyloid type detected</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Amyloid type not detected</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>53%</td>
<td>67%</td>
</tr>
</tbody>
</table>

DISCUSSION & CONCLUSIONS: The SRM-assay we provide here has potential to significantly improve the diagnostic workflow of systemic amyloidosis in the near future. With our assay we can not only reliably and correctly determine the main types of systemic amyloidosis using minimally invasive fat tissue aspirates, but we can also take advantage of the “amyloid signature” to generally detect amyloid deposition in those samples with high accuracy. If aspirates with little amyloid (1+ and 2+) cannot be typed directly, typing can be repeated after laser microdissection of Congo red-positive areas. After applying improvement measures to overcome some limitations, our approach could be implemented in routine SA diagnosis. Thereby it would significantly accelerate and facilitate the current workflow of diagnosing and typing of systemic amyloidosis.

REFERENCES: none
Antibodies targeting the N-terminal part of the amyloid β-peptide can impair lateral assembly of filaments and the intrinsic stability of amyloid fibrils

A Pamren1, I Iakovleva1, T Islam1, K Brännström1, L Sandblad2, A Olofsson1,*

1Department of Medical Biochemistry and Biophysics. Umeå University, SE-901 87, Umeå, Sweden
2Department of Molecular Biology, Umeå University, SE-901 87 Umeå, Sweden
Annelie.Pamren@umu.se

INTRODUCTION:
The self-assembly of the amyloid β-peptide (Aβ) into amyloid fibrils is linked to Alzheimer’s disease and factors affecting its assembly are consequently of interest to elucidate. It has recently been suggested that antibodies targeting the N-terminal part of the Aβ peptide could dissociate amyloid formations into monomers and low molecular weight oligomers [1]. We have explored this further and can here present an alternative explanation.

MATERIALS & METHODS:
We have studied how different types of both IgG and IgM antibodies can affect amyloid assembly by combining methods and techniques such as thioflavin-T analysis, Western Blotting, epitope mapping, and Transmission electron microscopy.

RESULTS:
We can here present an alternative explanation suggesting that the Aβ amyloid assembly in presence of antibodies affect the lateral assembly of filaments which result in thinner fibrillar structures with a lower ability to bind thioflavine T. A full effect is already seen at an Aβ: antibody ratio corresponding to around 150:1. Both IgM and IgG isotype antibodies can mediate the response which also render the fibrillar structure less stable. The location of the epitope strongly determines the strength of the effect. Antibodies targeting the N-terminal part of Aβ show a strong effect while antibodies targeting the middle region of the peptide show no effect. Interestingly, autoantibodies of the IgM isotype targeting Aβ is almost invariably present in all humans. Through mapping of the binding pattern from a few healthy individuals, we show that the intrinsic repertoire of anti-Aβ IgM antibodies may target several different Aβ epitopes on the peptide, including the N-terminal part.

DISCUSSION & CONCLUSIONS:
Many clinical trials regarding treatments of Alzheimer’s disease focus on vaccination strategies. When comparing antibodies targeting different regions of the Aβ peptide, we could see that while antibodies targeting the middle region show no effect on fibril formation, antibodies targeting the N-terminal part of the Aβ-peptide generate thinner fibrillary structures. A lateral dissociation of filament may provide a mode for destabilizing the amyloid structures and the specific spectra of clonal B-cell expansion may consequently be an important modulating factor of the fibrillar architecture and the pathology.

REFERENCES:
Serendipitous inhibition of Aβ and rVλ6Wil amyloid fibril growth by bi-functional peptides

JS Wall\textsuperscript{1,2}, A Williams\textsuperscript{1}, EB Martin\textsuperscript{1}, X Cheng\textsuperscript{3}, and SJ Kennel\textsuperscript{1,2}

\textsuperscript{1} Department of Medicine, University of Tennessee Medical Center, Knoxville, USA. \textsuperscript{2} Department of Radiology, University of Tennessee Medical Center, Knoxville, USA. \textsuperscript{3} Bioscience Division and Computer Science and Mathematics Division, Oak Ridge National Laboratory, Oak Ridge, USA.

jwall@utmck.edu

INTRODUCTION: Amyloidosis is characterized by the aggregation of partially misfolded proteins or peptides as fibrils that deposit in organs and tissues resulting in dysfunction and death. We have previously identified a series of amyloid-targeting synthetic polybasic peptides that specifically interact with amyloid fibrils \textit{in vitro} and deposits \textit{in vivo} \cite{Wall2011}. At present, there are no amyloid therapeutics that successfully prevent the growth or extension of amyloid fibrils. Indeed, the process of fibril growth post nucleation is widely considered unstoppable; however, decreasing the rate of fibrillogenesis or amyloid growth is an important intervention site and would represent another treatment option for patients with amyloidosis. We have developed novel, bi-functional amyloid binding peptides originally designed to enhance the lifetime of the circulation by the addition of albumin-binding peptide sequences to the amyloidophilic peptide p5. In fibrillogenesis assays, certain of these peptides have been shown, serendipitously, to inhibit fibril formation of two amyloidogenic precursors: Aβ(1-40) and recombinant human λ6 variable domain, Wil (rVλ6Wil). Molecular dynamics simulations suggest that they may act by capping the fibril end.

MATERIALS & METHODS: Crude preparations of bifunctional peptides and Aβ(1-40) were purchased from Anaspec (Fremont, CA). Recombinant Vλ6Wil was generated in \textit{E. coli} and purified from the periplasmic space using standard procedures. Fibrillogenesis assays were performed in phosphate buffered saline using 5 µM rVλ6Wil or 7.5 µM Aβ(1-40) in the presence of peptides at a 1:1 or 1:10 amyloid precursor to peptide molar ratio. The formation of fibrils was monitored by measuring the fluorescence emission of thioflavin T at 490 nm (excitation = 450 nm) over a 24 h period. Molecular dynamics simulations were performed as described \cite{Wall2015} using the Titan supercomputer at Oak Ridge National Laboratory.

RESULTS & DISCUSSION: Bifunctional peptides at 10-fold molar excess, or equimolar in certain cases, were able to delay the fibrillogenesis of both Aβ(1-40) and rVλ6Wil proteins at least 4-fold, from ~ 1-2 hours to more than 8 hours. Notably, non-bifunctional peptides had no effect on the lag time of fibrillogenesis. Peptides that were effective at inhibiting Aβ(1-40) were also inhibitors of rVλ6Wil, suggesting that there may be shared mechanisms of fibril formation. Computer modeling and molecular dynamics simulations of the peptide-Aβ fibril interaction indicated that the peptide may inhibit by “capping” the growing end of the fibril (Fig. 1). Based on these preliminary positive observations, we anticipate that these peptides may yield a novel theranostic reagent for the detection and inhibition of amyloid fibril growth.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{paper.png}
\caption{Molecular dynamics simulation of peptide “capping” a model Aβ fibril.}
\end{figure}

PROBING THE STRUCTURAL REQUIREMENTS OF POLYBASIC PEPTIDES FOR EFFECTIVE AND SPECIFIC AMYLOID REACTIVITY

JS Wall1,2, A Williams1, A Stuckey2, EB Martin1, T Richey1, C Wooliver1, RE Heidel3, X Cheng4 and SJ Kennel1,2

1 Department of Medicine, University of Tennessee Medical Center, Knoxville, USA. 2 Department of Radiology, University of Tennessee Medical Center, Knoxville, USA. 3 Department of Surgery, University of Tennessee Medical Center, Knoxville, USA. 4 Bioscience Division and Computer Science and Mathematics Division, Oak Ridge National Laboratory, Oak Ridge, USA.
jwall@utmck.edu

INTRODUCTION: Polybasic helical peptides, such as peptide p5, bind human amyloid extracts and synthetic amyloid fibrils [1]. When radiolabeled, peptide p5 and similar reagents, such as p5+14, have been shown to bind peripheral amyloid specifically in vivo thereby allowing imaging of the disease. At present, there are no FDA-approved clinical techniques for whole body imaging of systemic amyloidosis in patients. We hypothesize that understanding the features that govern amyloid binding will result in enhanced amyloid-targeting agents for imaging and therapy. The structural requirements for effective binding to heparan sulfate (heparin was used as a surrogate) and amyloid fibrils, the major polyelectrolytic components of amyloid, have been studied using in vitro analyses, SPECT/CT imaging, biodistribution and autoradiography.

MATERIALS & METHODS: Based on the structure of peptide p5, the following synthetic peptide homologs were evaluated: peptides with disrupted α-helices; a peptide with a propensity to form a β-sheet; and an all D-enantiomer variant of p5. Peptide-ligand interactions were studied using CD spectroscopy and solution-phase binding assays using radiolabeled peptides. The interaction of a subset of peptides was further studied by using molecular dynamics simulations. In vivo reactivity with systemic murine AA amyloid was assessed by SPECT imaging, tissue biodistribution measurements and autoradiography.

RESULTS: Disruption of the peptide α-helical structure accompanied reduced peptide binding to heparin and amyloid extracts. However, the all-D enantiomer and the β-sheet peptide bound all amyloid substrates as well as, or better than, p5. The interaction of α-helical and β-sheet structured peptides with Aβ fibrils was modeled and shown to involve both ionic and non-ionic interactions (see figure). In vivo, the β-sheet peptide specifically bound amyloid. The all-D enantiomer of p5 exhibited amyloid binding but also novel, intense, off-target reactivity in the renal cortex and hepatic sinusoids.

DISCUSSION: The α-helical secondary structure of peptide p5 is important for heparan/heparin and amyloid binding; however, helicity is not an absolute requirement for polybasic peptide-amyloid interactions as evidenced by the superior binding of a β-sheet peptide, both in vitro and in vivo. The all-D enantiomer of p5, despite binding ligands effectively in vitro, exhibited significant off-target reactivity in vivo and was, therefore, deemed unsuitable as an amyloid imaging agent. The polybasic β-sheet motif is a novel model structure for developing improved amyloid targeting reagents in vivo.

A NOVEL MURINE SYSTEM FOR VALIDATING THE SPECIFIC TARGETING OF PEPTIDES TO LIGHT CHAIN ASSOCIATED (AL) AMYLOID

SP Beierle, JS Foster, T Richey, A Stuckey, S Macy, SJ Kennel, and JS Wall

1 Department of Medicine, University of Tennessee Medical Center, Knoxville, USA. 2 Department of Radiology, University of Tennessee Medical Center, Knoxville, USA. 3 Department of Surgery, University of Tennessee Medical Center, Knoxville, USA.

INTRODUCTION: Light chain amyloidosis (AL) is the most common form of systemic amyloid disease. In the US, there are an estimated 4,000 new cases each year. Although novel immunotherapies are in clinical trials, the rapid development of effective treatments and novel imaging agents has suffered due to the lack of experimental animal models with amyloid-infiltrated organs. Our goal is to develop new targeting agents for AL amyloidosis that allow whole body imaging of patients for early diagnosis and disease monitoring. To assist with the validation of these imaging agents, we have recently developed a model of highly vascularized human AL amyloidoma in mice.

MATERIALS & METHODS: Human amyloid extracts were purified from autopsy-derived, amyloid-laden tissues. Synthetic amyloid fibrils were also generated using recombinant Vλ6Wil protein. To generate the intrahepatic or intrasplenic amyloidoma, mice were anesthetized with isofluorane and placed supine atop a warming pad and administered 125 µg of ketoprofen sc. The belly was clipped and cleaned before a 1 cm incision was made in the skin and abdominal wall. The liver or spleen was visualized and 200 µL (~0.6 – ~6 mg) of amyloid suspension injected into the organs using a 20 g needle. The abdominal wall and skin were then closed with one or two interrupted 5-0 monocryl stitches, and a running stitch of 5-0 monocryl, respectively. Skin glue was then applied to assure waterseal and avoid contamination of the wound. After 5 d, the distribution of 125I-labeled amyloidophilic tracers p5+14 and SAP was assessed by SPECT imaging and microautoradiography.

RESULTS: Injections of amyloids were well tolerated, and the mice recovered well. Intrahepatic injection was invariably more successful than splenic. Amyloid deposited within the liver became highly vascularized. Amyloid that extruded out of the injection site formed adhesions on the abdominal organ surfaces. Both the intrahepatic and adhesion amyloid deposits were labeled with 125I-p5+14 peptide, but not 125I-SAP, when given IV. Penetration of the 125I-p5+14 into the amyloid was dependent on the degree of vascularization and the size of the amyloid lesion – a binding site barrier effect was observed.

DISCUSSION: Injections of human AL amyloid extracts and rVλ6Wil fibrils resulted in pathology that was better vascularized than standard subcutaneous amyloidomas, allowing greater and more clinically relevant accessibility to targeting agents delivered IV. The amyloidomas bound 125I-p5+14 and validated the use of this agent for imaging AL amyloid deposits in patient organs. The binding of SAP to amyloid was possibly inhibited by an antibody response generated to SAP in the amyloid extract implanted 5 days previously. Although this model does not recapitulate aspects of the onset and progression of AL, it is a robust model of a mouse with vascularized AL amyloid in an abdominal organ than can be used for validating the efficacy of novel imaging agents, such as p5+14, in vivo.

Fig. 1: Synthetic λ6 fibrils surgically implanted into a mouse liver. Mouse was 5 days post-surgery. Original magnification 160x
PA30
Enthalpic forces correlate with selectivity of transthyretin-stabilizing ligands in human plasma

I Iakovleva1, K Bränström1, L Nilsson2, A Gharibyan3, A Begun2, I Anan4, M Walfridsson1, AE Sauer-Eriksson2, A Olofsson1

1 Department of Medical Biochemistry and Biophysics, Umeå University, 901 87 Umeå, Sweden. 2Department of Chemistry, Umeå University, 901 87 Umeå, Sweden. 3Department of Pharmacology and Clinical Neurosciences, Umeå University, 901 87 Umeå, Sweden. 4Department of public Health and Clinical Medicine, Umeå University, Umeå, Sweden.

irina.iakovleva@umu.se

INTRODUCTION:

The plasma protein transthyretin (TTR) is linked to human amyloidosis. Dissociation of its native tetrameric assembly is a rate-limiting step in the conversion from a native structure into a pathological amyloidogenic fold. Binding of small molecule ligands within the thyroxine-binding site of TTR can stabilize the tetrameric integrity and is a potential therapeutic approach. However, the clinical efficacy of a ligand can be limited by binding to blood plasma proteome. The nature of protein-ligand complex formation can be described by enthalpic and entropic energy contributions. The organisation of new chemical bonds requires a certain orientation of molecules in specific environment and will result in potential changes in enthalpy, \( \Delta H \) component. We therefore hypothesised that ligand possessing high \( \Delta H \) in the binding to its co-partner also would be more specific in complex environment.

MATERIAL & METHODS:

We have investigated nine potential TTR binders, according their stabilizing ability to TTR in presence and absence of human plasma. The binding constant (\( K_D \)), relative contributions of enthalpic (\( \Delta H \)) and entropic (\( \Delta S \)) forces were determined for all ligands using isothermal titration calorimetry (ITC). Efficacy in stabilizing TTR in human plasma was evaluated by urea-mediated denaturation assay.

RESULTS:

We shown how selectivity of TTR-stabilizing ligands can be significantly compromised in the presence other components. The binding strength between a particular ligand and TTR does not correlate well with its selectivity in plasma. We found that the enthalpic (\( \Delta H \)) component give better prediction for selectivity of drugs.

DISCUSSION & CONCLUSIONS:

Through analysis of the thermodynamic signature using isothermal titration calorimetry we discovered a better correlation between selectivity and the enthalpic component of the interaction. This is of specific interest in the quest for more efficient TTR stabilizers, but a high selectivity is an almost universally desired feature within drug design and the finding might have wide-ranging implications for drug design.
RECRUITMENT OF HUMAN LIGHT CHAIN PROTEINS BY SYNTHETIC FIBRILS IS DEPENDENT ON DISEASE STATE AND MAY BE USED TO PREDICT AMYLOIDOGENIC PROPENSITY

EB Martin¹, A Williams¹, C Wooliver¹, RE Heidel³, S Adams³, J Dunlap⁴, M Ramirez-Alvarado⁵, L Blanca-Mejia⁵, SJ Kennel¹,² and JS Wall¹,²

¹ Department of Medicine, University of Tennessee Medical Center, Knoxville, USA. ² Department of Radiology, University of Tennessee Medical Center, Knoxville, USA. ³ Department of Surgery, University of Tennessee Medical Center, Knoxville, USA. ⁴ Microscopy and Imaging Center, University of Tennessee, Knoxville, USA. ⁵ Departments of Biochemistry and Molecular Biology, and Immunology, Mayo Clinic, Rochester, USA

emartin@utmck.edu

INTRODUCTION: Multiple myeloma (MM) and light chain (AL) amyloidosis are plasma cell dyscrasias characterized by the presence of serum free monoclonal immunoglobulin light chain (LC) proteins. In both cases, the LC may aggregate and form amorphous renal casts or well-structured amyloid fibrils deposited systemically in patients with MM and AL, respectively. The overall survival for patients with AL is generally worse than for those with MM. Approximately 15% of MM patients will also develop LC amyloidosis. Despite intensive efforts, the precise mechanism underlying amyloid growth in vivo remains enigmatic. We have studied the recruitment of both AL and MM LC proteins, derived from patient urine, by synthetic rVλ6Wil fibrils. Furthermore, the assay permitted statistical differentiation of AL from MM patients.

MATERIALS & METHODS: Purified urinary LC proteins were radiolabeled with iodine-125 using a standard oxidation reaction with chloramine T, followed by purification using size exclusion chromatography. The radiopurity was assessed by SDS-PAGE and phosphorimaging. A solution-phase pull down assay was used to measure the recruitment of radiiodinated LC (~5 ng) by rVλ6Wil fibrils (25 μg) after 1 h, 3 h and 24 h of incubation. Immunogold electron microscopy using a κ LC-reactive antibody was used to visualize a certain κ LC bound to fibrils. A preliminary assay to discern benign from amyloidogenic LC has been established using ¹²⁵I-rVλ6Wil.

RESULTS: Electron microscopy revealed the preferential accumulation of AL LC on rVλ6Wil fibrils at the fibril ends rather than randomly dispersed throughout fibril aggregates (Fig. 1, left). Our data further indicated that both AL and MM patient-derived LC proteins were recruited by rVλ6Wil fibrils; however accumulation of AL LC was significantly higher (p < 0.005) than MM proteins at all time points studied (Fig. 1, right). The assay was able to accurately discern MM from AL-derived LC proteins. Notably, one LC from a MM patient who developed AL two years after initial diagnosis, behaved as an AL protein in the assay.

DISCUSSION: We have studied the recruitment of intact, patient-derived LC proteins by synthetic amyloid fibrils composed of variable domain fragments, as found in patient-derived amyloid samples. Although both LC derived from both AL and MM patients were recruited by the fibrils, the assay was able to discriminate between AL- and MM-derived LC. This observation led to the development of a preliminary test to discern amyloidogenic LC from benign counterparts. This capability may be useful in discerning those patients with multiple myeloma or monoclonal gammopathy of unknown significance who are at risk of developing amyloidosis based on LC recruitment competency.

Fig. 1: Binding of anti-κ mAb to κLC associated with rVλ6Wil fibrils (left). Recruitment of AL (circle) and MM (square) LC by rVλ6Wil fibrils at 3 time points (right).
Heparan sulfate is involved in cellular uptake of alpha-synuclein amyloid fibrils but not soluble non-fibrillar oligomers

E Ihse¹, E Masliah², JD Esko³

¹Department of Public Health and Caring Sciences, Uppsala University, Uppsala, Sweden.
²Department of Neurosciences, University of California, San Diego, USA.
³Department of Cellular and Molecular Medicine, University of California, San Diego, USA.

INTRODUCTION: Spreading of the pathology to increasingly larger areas of the brain in neurodegenerative diseases like Alzheimer’s and Parkinson’s disease has been proposed to be caused by a prion-like seeding mechanism. It has been shown that protein aggregates involved in these diseases can transfer from one cell to another, but not much is known about how the internalization occurs. Glycosaminoglycans (GAGs) are sulfated sugar polymers that are abundant on cell surfaces and in the extracellular matrix, that are known to interact with protein aggregates that have an amyloid conformation. Especially the GAG subgroup heparan sulfate seem to have a propensity to bind to amyloid, as it has been found in protein deposits of essentially every amyloid disease regardless of the aggregating protein. A few studies also implicate interactions between amyloid and another GAG subgroup called chondroitin sulfate. It has also recently been shown that heparan sulfate is involved in the cellular uptake of aggregates of A-beta, tau and prion protein, proteins that form amyloid deposits in Alzheimer’s disease and prion diseases. The objective of the present project was to investigate if heparan and chondroitin sulfate are involved in cellular internalization of the Parkinson’s disease related protein alpha-synuclein (α-syn), and if this is true only for aggregates with an amyloid fibril conformation or also for soluble non-fibrillar oligomeric species.

MATERIAL & METHODS: Rat neuroblastoma B103 cells were treated with α-syn in oligomeric or amyloid fibril conformation and effects on cell surface binding/uptake by the addition of heparin and heparinas as well as chondroitin sulfate and chondroitinaseABC to the cell media was determined by an α-syn sandwich ELISA. Furthermore, uptake of α-syn amyloid fibrils labelled with pHrodo, a pH-sensitive dye the only fluoresce once inside the endo-lysosomal pathway, was studied by flow cytometry on CHO cells deficient in different GAG synthesis enzymes. Confocal microscopy was used to study possible colocalization of heparan sulfate and α-syn amyloid fibrils.

RESULTS: Cellular binding/uptake of α-syn amyloid fibrils was almost completely abolished by the addition of heparin and heparinases to the cell media of B103 cells, while binding/uptake of oligomers was only slightly inhibited. Addition of chondroitin sulfate and chondroitinase to the cell media on the other hand, had only slight to no effect on the uptake of α-syn amyloid fibrils. Studies on CHO cell mutants show that GAGs are very important for uptake of α-syn amyloid fibrils, and that heparan sulfate is the most important subgroup for this process, while chondroitin sulfate is only slightly involved. Results from CHO cell mutants also indicate that the interaction is dependent on the general degree of sulfation of heparan sulfate, while reducing specific sulfation sites, like 2-O sulfation, has no to little effect.

DISCUSSION AND CONCLUSIONS: Hindering transfer of α-syn amyloid seeds from one cell to another can possibly diminish spread of the pathological state to larger areas of the brain in neurodegenerative diseases. At the moment, knowledge of the actors involved in aggregate transfer between cells is sparse, and studies are needed to identify targets for therapeutic interventions to hinder such propagation. In this study, we show that heparan sulfate is involved in the cellular uptake of some, but not all types of α-syn aggregates, and the amyloid fibrillar conformation is likely the determinant for such involvement. Further studies should be conducted to understand the specifics of the interaction between cell surface GAGs and α-syn fibrils, as using agents to hinder this specific interaction without disturbing heparan sulfates many other interactions and functions may be a useful future therapy for diseases where amyloidotic aggregation of α-syn, as well as other proteins, are found.
Unraveling amyloid formation and structure at single aggregate scale by infrared nanospectroscopy

FS Ruggeri\textsuperscript{1,2}, Sophie Vieweg\textsuperscript{3}, Giovanni Longo\textsuperscript{2}, Hilal Lashuel\textsuperscript{3}, Giovanni Dietler\textsuperscript{2}, Tuomas Knowles\textsuperscript{2}

\textsuperscript{1} Department of Chemistry, University of Cambridge, United Kingdom. \textsuperscript{2}Laboratory of Physics of Living Matter, EPFL, Switzerland. \textsuperscript{3} LMNN, EPFL, Switzerland

fsr26@cam.ac.uk

INTRODUCTION:

Amyloids are insoluble proteins aggregates implicated in the onset of several neurodegenerative disorders, such as Alzheimer’s, Huntington’s and ataxia diseases. During aggregation, monomeric proteins undergo internal structural rearrangement forming amyloid fibrils with a universal cross $\beta$-sheet quaternary structure. This structure is independent by the monomeric initial native protein and it is the fingerprint of the onset of the related disease.

MATERIAL & METHODS:

Infrared nanospectroscopy (nanoIR) is an innovative tool that exploits the combination of two techniques commonly used to study protein aggregation: Atomic Force Microscopy (AFM) and infrared spectroscopy (IR). The first can provide information on the morphology and mechanical properties of the species formed along the aggregation pathway, the second can characterize conformational changes in protein secondary structure. Although useful, these conventional techniques do not tell us separately if/at which time point misfolding occurs, nor what is the secondary structure of the individual species. Their combination, in infrared nanospectroscopy, enables a structural characterization at the nanoscale of the formation and properties of amyloids through the acquisition of nanoscale chemical IR maps or spectra.

RESULTS:

First, we could characterize at the individual aggregate scale the conformational rearrangements of proteins during their misfolding and aggregation. Furthermore, combining nanoIR with conventional AFM nanomechanical mapping, we correlated the secondary structure of amyloid intermediates and final aggregates to their nanomechanical properties. Our results directly demonstrated, for the first time at the individual amyloid species scale, that the increase of $\beta$-sheet content is a fundamental parameter determining amyloids intrinsic stiffness.\textsuperscript{1,2} As next step, we structurally characterized single huntingtin amyloid fibrils as a function of the length of their mutated polyglutamine (polyQ) stretch. The results demonstrated that fibrils with higher polyQ content had a higher quality (i.e. intermolecular hydrogen bonds) of amyloidogenic $\beta$-sheet structure. Finally, it was shown that it exists a direct correlation between huntingtin polyQ stretching size and age of Huntington’s disease onset. Thus, this data strongly suggests the structure improvement as one of the main factors causing toxicity above the polyQ pathogenic threshold for the disease onset.

DISCUSSION & CONCLUSIONS:

Elucidating the formation and the structural properties of amyloidogenic proteins is essential for the unraveling of the molecular basis of their function in health and disease. Indeed, the improved structure could be more efficient in damaging cellular membranes, in sequestrating transcription factors, thus impairing the transcription of essential genes or could more easily overwhelm the ubiquitin-proteasome system, leading to its failure. The comprehension of these mechanisms is central to develop new pharmacological approach to neurodegenerative disorders.

Lipid membrane induced α-synuclein aggregation

J Pallbo¹, R Gaspar¹, A Dabkowska¹, S Linse², U Olsson¹, E Sparr¹

¹ Department of Physical Chemistry, Lund University, Lund, Sweden. ² Department of Biochemistry and Structural Biology, Lund University, Lund, Sweden.

jon.pallbo@gmail.com

INTRODUCTION: It has been shown that the aggregation of the Parkinson’s disease associated protein α-synuclein into amyloid fibrils can be accelerated by the presence of lipid membranes in vitro, with a strong dependence on the membrane lipid composition. Specifically, accelerated aggregation has been observed in the presence of anionic model vesicles containing ganglioside lipids, or in the presence of exosomes extracted from neural cells, which have gangliosides as a normal constituent. Other anionic lipids do not cause accelerated aggregation [1]. It was hypothesized that the difference in aggregation kinetics was related to differences in the adsorption of α-synuclein to membranes containing gangliosides versus membranes containing other anionic lipids. To test this, we are in the present study investigating the adsorption of monomeric α-synuclein to model membranes with different compositions.

MATERIAL & METHODS: We have used membranes composed of the zwitterionic lipid DOPC, the anionic lipid DOPS, and the anionic ganglioside lipid GM1. The adsorption has been measured using fluorescence correlation spectroscopy (FCS), and membrane induced conformational changes of α-synuclein has been measured using circular dichroism (CD) spectroscopy.

RESULTS: α-synuclein adsorbs strongly to membranes containing DOPS or GM1, and so far we have not observed any significant differences in either the extent of adsorption or in the conformational change of α-synuclein upon adsorption to membranes containing DOPS and GM1.

DISCUSSION & CONCLUSIONS: There appears to be no difference in adsorption or conformational change of α-synuclein upon binding to membranes containing DOPS and GM1, despite the fact that membranes containing GM1 cause accelerated α-synuclein aggregation, whereas membranes containing DOPS do not cause accelerated aggregation. This suggests that there is no simple relationship between membrane binding of monomeric α-synuclein and membrane induced α-synuclein aggregation.

Modelling cardiac ATTR amyloidosis

Centre for Amyloidosis and Acute Phase Proteins, Division of Medicine, UCL, London, UK.
p.simons@ucl.ac.uk

INTRODUCTION
Cardiac ATTR amyloidosis is an under-recognised cause of morbidity and mortality affecting the elderly. To date, no good models of cardiac ATTR amyloidosis have been reported. The rare TTR52P variant is aggressively amyloidogenic, and is the cause of a highly penetrant adult-onset form of autosomal dominant familial amyloidosis, in which cardiac amyloidosis is a prominent feature. The recent characterization of this variant provided a new possibility for modeling cardiac ATTR amyloidosis.

MATERIALS & METHODS
Transgenic mice were generated on the C57BL/6 background by pronuclear microinjection. hTTR52P transgenic mice were bred with wild-type mice, or crossed with TTR knockouts. Human TTR was assayed by electroimmunoassay using anti-hTTR antibody (Dako). Western blots of serum proteins were probed with anti-TTR antisera (Dako or Binding Site). Amyloid deposition was assessed by Congo red staining and polarizing microscopy. Amyloid fibril type was assessed by immunoperoxidase staining using anti-TTR (Dako) anti-SAA (R&D systems) and anti-apoAII (a kind gift of Prof. Higuchi).

RESULTS
The concentrations of hTTR were assayed in sera of hemizygous hTTR52P transgenic mice, and measured to be 1.6 ± 0.14 mg/mL (mean ± SD) in males, and 1.1 ± 0.18 mg/mL in females. By Western blot analysis of transgenic mouse serum alongside normal human serum, an additional slower migrating minor band was consistently observed in transgenic mouse serum, which was abolished by PNGase F treatment.

Congo red staining revealed no amyloid in 22 different tissues in untreated transgenic mice of ages up to 22 months. In contrast, amyloid deposits were found in mice injected 4-7 months previously with splenic amyloid from a hTTR52P patient. Among 29 mice analysed, amyloid was detected in 22, including 17 with cardiac amyloid. In each case, there was positive immune histochemical staining for human TTR in the amyloid deposits (Fig. 1), and no staining for apoAII or SAA. No difference was apparent in amyloid deposition between mice wild-type, heterozygous or knockout for the endogenous TTR. Notably, cardiac amyloid was present in 16 of 17 amyloid-seeded transgenic males, but only 1 of 12 females.

DISCUSSION
The transgenic mice reported here express hTTR52P variant at concentrations comparable to those in humans. Higher concentrations were measured in males than in females, as is the case in people. The minor glycosylated component is consistent with misfolding of nascent protein in the secretory pathway, and tagging for ER-associated decay. Despite this, and the early onset of amyloidosis in hTTR52P patients, no amyloid deposition was detected in untreated transgenic mice. In contrast, amyloid was consistently observed in mice of similar or younger ages that had been seeded with patient-derived amyloid. This suggests that endogenous formation of hTTR52P amyloid seeds is limiting in mice. A further striking observation was the much greater incidence of amyloid in transgenic males than in females, which might reflect the large male preponderance of clinical cardiac ATTR amyloidosis. Although the amounts of amyloid in these mice were modest, these mice promise to be invaluable for investigating the pathogenesis of cardiac ATTR amyloidosis as well as for evaluating therapies and diagnostic methods.
PA36
Development of peptidomimetic probes specific for non-native oligomeric transthyretin for the detection and isolation of transthyretin oligomers from patient plasma

JD Schonhoft1,2, C Monteiro1,2, J Kelly1, L Plate1, C Parker1, Y Eisele1, J Dendle1, E Powers1, JW Kelly1

1 Departments of Chemistry and Molecular and Experimental Medicine,
The Scripps Research Institute, La Jolla, California, USA

2 Equal contributions
jschonho@scripps.edu

INTRODUCTION:
Increasing evidence supports the hypothesis that the soluble ensemble of non-native oligomeric or prefibrillar protein aggregates is a pathological driver of amyloid diseases, along with amyloid fibrils. Despite the apparent contribution of non-native oligomers as drivers of degenerative pathology, chemical tools to detect non-native oligomers in patients are limited. This deficiency has hampered progress in understanding the relationship between non-native transthyretin (TTR) oligomers, cell/tissue toxicity and amyloid disease progression.

RESULTS:
Protein aggregation and subsequent amyloid fibril formation are processes characterized by a rough free energy landscape that features kinetic traps, and thus stable on and off pathway intermediates. In such a landscape, non-native oligomeric conformations of TTR are unlikely to be well-packed structures. Thus, we hypothesized that non-native TTR oligomers would contain defect sites that could be targeted using fluorescently labelled peptides. It was anticipated that these peptides would not recognize natively folded TTR or the amyloid fibril conformations that are both efficiently packed and are likely devoid of binding sites resulting from structural defects. We have developed a novel approach using peptides and peptidomimetics to target non-native TTR structures at intermediate stages in the amyloido genesis cascade. Furthermore, we have turned these lead molecules into photo-affinity probes and have begun to establish their targets in plasma samples from ATTR patients, leading to the discovery of new putative biomarkers for these diseases.

DISCUSSION & CONCLUSIONS:
We envision that this strategy will be general for discovering peptide-based conformation-specific probes of amyloidogenic proteins and expect our current set of probes to be useful in understanding the underlying mechanisms of the TTR amyloidoses and the mechanism of tissue tropism.
Scrutinizing the oligomer proteotoxicity hypothesis in the transthyretin (TTR) amyloidoses – characterization of novel, highly stable and cytotoxic TTR oligomers

YS Eisele\textsuperscript{1,2}, L Plate\textsuperscript{1,2}, GJ Morgan\textsuperscript{1,2}, GC Lander\textsuperscript{3}, N Reixach\textsuperscript{2}, JN Buxbaum\textsuperscript{2}, RL Wiseman\textsuperscript{2,4}, E Powers\textsuperscript{1}, JW Kelly\textsuperscript{1,2,5}

\textsuperscript{1}Department of Chemistry, \textsuperscript{2}Department of Molecular and Experimental Medicine, \textsuperscript{3}Department of Integrative Structural and Computational Biology, \textsuperscript{4}Department of Chemical Physiology, \textsuperscript{5}The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, California 92037, USA.

eisele@scripps.edu

INTRODUCTION: Transthyretin (TTR) is one of over 30 amyloidogenic proteins that are known to misfold and form amyloid fibrils. There is both genetic and pharmacologic evidence that the process of amyloid fibril formation causes age-related TTR degenerative diseases [1]. The amyloid load does not appear to change in patients that respond to tafamidis, a small molecule that prevents TTR amyloidogenesis by kinetically stabilizing the native TTR tetramer [2]. Thus, smaller, non-native TTR oligomers are hypothesized to be a driver of the degenerative pathology [1]. Very little is yet known about whether and how non-native TTR oligomers form in the context of the TTR amyloidoses.

MATERIALS & METHODS: Here, we report the time- and concentration-dependent formation of TTR oligomers from monomeric TTR as well as from destabilized initially tetrameric TTR variants at near physiological pH at 37°C. Oligomers were biochemically and biophysically characterized in regard to their molecular weight, stability, and ligand binding. Atomic force microscopy (AFM) and electron microscopy (EM) provided information on oligomer morphology and structure. Toxicity of the oligomers was assessed in a \textit{C. elegans} pharyngeal pumping assay [3].

RESULTS: TTR oligomer formation requires the dissociation of the native TTR tetramer, likely involves partial misfolding, and compromises the small molecule-binding site present in the native TTR tetramer. Interestingly, once formed, the oligomers seem relatively stable and their stability further increases over time, probably owing to conformational annealing. AFM and negative-stain EM reveal a filamentous, protofibrillar appearance. Moreover, these TTR oligomers show cytotoxicity in a \textit{C. elegans} pharyngeal pumping assay.

DISCUSSION & CONCLUSIONS: All these traits indicate that the newly characterized, highly stable and cytotoxic non-native TTR oligomers might be highly relevant in the TTR pathogenesis cascade. Ongoing studies focus on deciphering the precise structure of these TTR oligomers and delineating a structure-toxicity relationship.

REFERENCES:


A method for the early diagnosis of the transthyretin amyloidoses using non-native oligomeric transthyretin-specific peptidomimetic probes

C Monteiro1*, JD Schonhoft1*, J Kelly1, L Plate1, M Novais2, Y Eisele1, J Dendle1, E Powers1,
T Coelho2, JW Kelly1

1 Departments of Chemistry and Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California, USA, 2 Unidade Corino de Andrade, Hospital de Santo Antonio, Porto, Portugal. *Equal contributions

INTRODUCTION: The transthyretin (TTR) amyloidoses are a group of clinically heterogeneous diseases that are often misdiagnosed or diagnosed late in their clinical course1,2. Available diagnostic methods rely heavily on the presence of amyloid fibrils or organ damage, both indicators of an advanced disease process. The need for non-invasive early diagnostic methods is highlighted by the finding that currently available therapies for these diseases (i.e., liver transplant and kinetic stabilizers) have proven to be much more effective when used early3. Therefore, we hypothesize that the development of probes that specifically detect and quantify soluble non-native TTR oligomers that precede the formation of amyloid fibrils, and presumably are present in patient plasma, will lead to the development of early diagnostic methods for these diseases.

RESULTS: Using rational design, we have identified several peptides that incorporate specifically into non-native oligomeric TTR structures derived from recombinant TTR. We have also tested these same probes with plasma from symptomatic familial amyloid polyneuropathy patients and wild-type transthyretin cardiomyopathy patients in order to determine the ability of the peptide probes to detect non-native TTR oligomers ex vivo, i.e., in blood plasma samples. One of our probes nicely distinguishes symptomatic patients (wild-type TTR cardiomyopathy and Val30Met polyneuropathy, FAP) from asymptomatic Val30Met mutation carriers and healthy controls with a wild-type TTR genotype (Figure 1).

DISCUSSION & CONCLUSIONS: We developed a non-native TTR oligomer probe into a quantifiable diagnostic method, amenable for use in the clinical setting. Further work is being done to explore the nature of the oligomers detected in patient plasma and to validate this diagnostic strategy. We envision that this approach will be useful not only for early diagnosis and in timing the start of disease-modifying therapies, but also for basic science purposes in terms of understanding the underlying mechanisms of the TTR amyloidoses and their tissue tropism.

Soluble β-amyloid peptide affecting myosin Vb mediated GLUT4 traffic in cultured neurons.

Oliveira, L.T.1,4; Leon, G.V.O.4; Provance, D.W.2; de Mello, F.G.3; Sorenson, M.M.1; Salerno V.P.1,4

1Instituto de Bioquímica Médica - UFRJ; 2Centro Desenvolvimento Tecnológico de Saúde - IOC; 3Instituto de Biofísica Carlos Chagas Filho - UFRJ; 4Departamento de Biociências da Atividade Física - EEFD / UFRJ / Rio de Janeiro / Brasil.

Introduction: Numerous studies have shown that diabetes is a risk factor for spontaneous Alzheimer’s disease. While these studies suggest that diabetes can contribute to Alzheimer’s disease, the implications of AD on diabetes are practically unexplored. The classic mediator of the pathophysiological effects, Aβ42 peptide, has been shown to enter neurons and lead to an alteration of the intracellular distribution of the molecular motor myosin Vb. Myosin Vb functions in memory and learning by participating in the strengthening of the long-term potentiation (LTP) of synaptic transmissions. It has also been implicated in the translocation of the glucose transporter, GLUT4, to the plasma membrane in response to insulin, a process that is defective in diabetes.

Material and methods: Here we examined the impact of Aβ42 on intracellular transport of GLUT4 transporter by MVb in chick retinal neurons. Cultured cells were maintained 4 days in vitro (DIV) at low density, then challenged with fluo-Aβ42 oligomers for 30 min and prepared for fluorescence and differential interference contrast (DIC) microscopy.

Results: Previously, we showed that Aβ42 labeled oligomers internalized for an extended time (more than 30 min) display a punctate distribution that shifts from peripheral neuronal processes to the perinuclear region. Our immuno-cytochemistry assays revealed an impairment of GLUT4 transporter and a loss of these receptor exposed at the cell membrane and this loss could affect synaptic activity.

Discussion and Conclusions: The results suggest an alteration in distribution and a reduced level at the cell surface, as well as an increased colocalization with myosin Vb, which can partially explain the changes in glucose metabolism associated with AD. It is also shown that the presence of the Aβ40 peptide inhibits the internalization of the Aβ42 peptide in cultured cells. Together, the results provide additional targets for the development of therapeutics against the progression and effects of Alzheimer’s disease.
Engineered binding proteins to islet amyloid polypeptide and other amyloidogenic intrinsically disordered proteins

EA Mirecka¹, S Feuerstein², L Gremer¹², G Schröder¹², M Stoldt¹², D Willbold¹², W Hoyer¹²

¹ Institute of Physical Biology, Heinrich Heine University, 40225 Düsseldorf, Germany. ² Institute of Complex Systems (ICS-6), Structural Biochemistry, Research Centre Jülich, 52425 Jülich, Germany.

INTRODUCTION: Engineered binding proteins targeting amyloidogenic intrinsically disordered proteins aid in the elucidation of the amyloid formation mechanism and suggest therapeutic strategies. We have developed binding proteins, termed β-wrapins (β-wrap proteins), with nanomolar affinity for monomers of disease-related proteins such as islet amyloid polypeptide (IAPP), tau, and α-synuclein [1-3]. To gain structural and mechanistic insight into the inhibition of IAPP amyloid formation, we characterize here the interaction of IAPP with the β-wrapin HI18.

MATERIAL & METHODS: We have determined the structure of IAPP in complex with HI18 by NMR spectroscopy. Biophysical experiments and cell viability assays were employed to characterize the inhibitory effect of HI18 on IAPP amyloid formation and toxicity.

RESULTS: The NMR structure of the IAPP:HI18 complex reveals a β-hairpin conformation of IAPP, containing two β-strands that correspond to two amyloidogenic motifs, connected by a turn established around Ser-20. HI18 stabilizes the IAPP β-hairpin motif by, inter alia, enclosing the aromatic amino acids Phe-15 and Phe-23 in the core of the complex. We find that HI18 inhibits IAPP aggregation and toxicity at low substoichiometric concentrations.

DISCUSSION & CONCLUSIONS: This study highlights how the conformational preferences of IAPP can be targeted to achieve inhibition of amyloid formation. Our results reveal a common preference of IAPP and other amyloidogenic proteins for formation of β-hairpin motifs and demonstrate a critical role of hairpin conformers [3,4] in the control of amyloid formation.

The human systemic amyloid precursor transthyretin (TTR) is a multimodal suppressor of Aβ amyloidogenesis

X. Li¹, Y. Song², K. Garai³, C. Sanders², R. Pappu³, J. N. Buxbaum¹

¹The Scripps Research Institute, ²Vanderbilt University, ³Washington University in St. Louis

jbux@scripps.edu.

INTRODUCTION: TTR is a human systemic amyloid precursor and as a misfolded ER cargo it is subject to the hepatic UPR. However, neurons increase its production in human AD under the influence of HSF1. In transgenic mice highly over-expressing human wild type TTR and FAP patients with peripheral tissue deposition, TTR deposits are not seen in brain parenchyma although they may be present in vessels and the leptomeninges, suggesting that TTR expression is cell type dependent and highly regulated in neurons. In vivo TTR suppresses the behavioral and neuropathologic changes in the APP23 transgenic model of human AD. Neuronal TTR is also increased in human Parkinson’s disease, human α-synuclein expressing mice, PrP infected mice, human HIV encephalitis and human HIV gp120 transgenic rats.

MATERIALS & METHODS: We have used in vitro assays of fibrillogenesis, nuclear magnetic resonance spectroscopy, fluorescence correlation spectroscopy and assays of protein protein interaction in transfected cultured cells to examine the mechanism of the inhibition of Aβ fibril formation.

RESULTS: The interaction between various TTR conformers and amyloidogenic substrates is complex. The TTR tetramer binds Aβ monomer through Aβ residues 17-21, inhibiting Aβ oligomerization, fibrillogenesis and cytotoxicity. In vitro and in cells it also binds the β-secretase fragment β-CTF (C99) at amino acids G659, A665 and T668. In cultured cells TTR binding inhibits C99 phosphorylation and its cleavage by γ-secretase thus reducing Aβ production. The TTR residues involved in the interaction with C99, like those involved in Aβ binding are in, or adjacent to, the hydrophobic T4 binding region of the tetramer, which behaves as a conformation specific hydrophobic interaction region for both small molecules and hydrophobic portions of polypeptide chains. Ligand specificity and affinity are determined by specific amino acids. The interactions increase TTR tetramer stability, reducing its own tendency toward monomer release and amyloid formation.

An engineered TTR monomer (F87M/L110M) [MTTR] that cannot form tetramers is highly amyloidogenic but it is also a more potent inhibitor of Aβ fibrillogenesis in vitro than the native TTR tetramer. Fluorescence correlation spectroscopy (FCS) and fluorescence burst analyses revealed that the mechanism of inhibition of fibril formation involves the sequestration of Aβ oligomers by MTTR oligomers, acting as a sink for the species that become the source of primary and/or secondary nuclei that catalyze fibril formation. The reaction detours Aβ molecules into large, amorphous, co-aggregates with TTR, which prevent their participation in fibril formation and cytotoxicity.

CONCLUSIONS: Thus while TTR is pathogenic as an amyloid precursor, in neurons it appears to behave as a multi-functional stress responsive molecule with chaperone-like properties for proteins with exposed hydrophobic regions.
PA42

TRANSFORMATION OF MESENCHYMAL STEM CELLS (MSCs) REPRESENTS AN IMPORTANT PROCESS IN THE REPAIR OF THE DAMAGED MESANGIUM

BACKGROUND: Many investigators have supported the idea that MSCs participate in the process of repair / regeneration exclusively by providing paracrine factors that enhance the process. Using a model of mesangial damage produced by glomerulopathic immunoglobulin light chains resulting in mesangial matrix replacement in AL-amyloidosis, the role of MSCs in the repair process was investigated.

MATERIALS AND METHODS:
In-vitro and ex-vivo experimental platforms were used to address the issue. A 6 dimensional (6D) live cell was the in-vitro system used. Mesangial cells (MCs) were incubated with light chains obtained from the urine of patients with renal biopsy-proven AL-Am. Similar amyloidogenic light chains were perfused through the renal artery in the ex-vivo platform. The respective lesions were reproduced in both –platforms. Then, tagged MSCs were introduced. Immunofluorescence, immunohistochemistry and electron microscopy were used to evaluate samples obtained at different time frames. Stains for smoothelin, muscle specific actin, smooth muscle actin, CD68 and were used to monitor phenotypic transformation of MSCs in the process of repair.

RESULTS:
MSCs transformed from an undifferentiated to a macrophage phenotype to clear the damaged mesangial areas. The process showed transformed MSCs phagocytosing cellular debris resulting from apoptotic mesangial cells and damaged matrix elements, and amyloid fibrils. After the cleaning process was finished, MSCs acquired morphologic and immunophenotypic characteristics of MCs as they proceeded to lay down new mesangial matrix.

CONCLUSIONS:
MSCs manifest great plasticity as they proceed to repair the damaged mesangium in AL-Am. The fact that they transform to a macrophage phenotype followed by transformation to MCs allows them to perform different crucial functions during the process of repair. The restored mesangium is possible as new MCs derived from MSCs are able to reproduce the normal mesangium.
Beyond current detection methods: Urinary exosomes allow for the identification of pathogenic light chains in light chain amyloidosis tissues.

Marina Ramirez-Alvarado1,2, David R. Barnidge3, Angela Dispenzieri4, Marta Marin-Argany1, Christopher J. Dick1, Samih H. Nasr3, Christopher J. Ward6, and Nelson Leung4,5.

1Department of Biochemistry and Molecular Biology, 2Department of Immunology, 3Department of Laboratory Medicine and Pathology, 4Division of Hematology, 5Division of Nephrology and Hypertension, Mayo Clinic, 200 First St. SW, Rochester, MN 55905, USA. 6The University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160

Background: Immunoglobulin light chain (AL) amyloidosis is a potential fatal complication of B-cell clonal proliferation. Currently, the best biomarker for treatment monitoring is serum free light chain (FLC) assay. The FLC assay has been shown to be better than serum and urine immunofixation but it cannot distinguish monoclonal FLC from polyclonal FLC once it drops below the lower limit of normal for FLC, which has implications regarding therapeutic decision making.

Urinary exosomes (EXs) are the smallest members of the extracellular vesicle family that are excreted in the urine. They are formed from the invagination of endosomal membranes on the cell surface. These endosomes fuse with the larger multivesicular body (MVBs). The EXs are released when the MVB fuses with the plasma membrane and the internal vesicles are discharged. EXs contain cytoplasm, intracellular proteins, nucleic acids, and plasma membrane proteins in the right topology. EXs are suspected to be involved with cellular communication and waste removal. Urinary EXs have previously been found to display different characteristics among patients with AL amyloidosis, multiple myeloma (MM), and monoclonal gammopathy of undetermined significance (MGUS). High molecular weight LC oligomers are found only in patients with active AL amyloidosis (1).

Hypothesis: We hypothesize that urinary EXs can be used as a biomarker to assess renal response in cases where a patient reaches hematologic complete response but continues to exhibit organ progression.

Methods: Four patients at different stages of AL were selected for this study. EXosomes were extracted and fractionated as previously reported (1). Oligomeric light chains and glomerular EX were identified using western blotting. Intact immunoglobulin light chains were identified in patient plasma, EX, and kidney biopsy amyloid deposits using mass spectrometry as the detection method (2).

Results: Patient clinical parameters and disease status are listed in Table 1. Oligomeric LCs species were found in urinary EXs of patient AL-ex11 with normal FLC ratio, negative bone marrow biopsy, 3.4 g/d of proteinuria and progressive renal failure. Urinary EXs from patients AL-ex12, AL-ex13 and AL-ex14 did not contain oligomeric LCs. Patients AL-ex13 was in hematologic and organ CR while AL-ex14 was in hematologic response but incomplete renal response. Two immunoglobulin light chain species (lambda 6a (IGLV 6-57) and lambda 4/5) were identified in AL-ex11 urinary EXs and plasma by mass spectrometry. The lambda 6a LC found in the urinary exosomes has the same molecular mass and sequence of the protein found in the kidney amyloid biopsy and the cDNA from the original plasma cell clone. Sequencing for the lambda 4/5 clone is ongoing. This documentation of a continued presence of a pathogenic light chain helped explain the clinical deterioration of patient AL-ex11. Patients with complete hematologic and organ response lacked pathogenic light chains in the EXs and/or the plasma. The urinary EXs enrich the pathogenic protein and allowed for the identification of the pathogenic light chain protein by mass spectrometry and cDNA sequencing.

Conclusion: Urinary EXs could be an essential tool to assess renal progression/response in AL amyloidosis.

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<th>Immunofixation</th>
<th>FLC ratio</th>
<th>Hem Response</th>
<th>Scr response</th>
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References:
Study of the conformational changes occurring in human transthyretin that are necessary for amyloid fibril formation in disease and for its role as a detoxifier.

Seyyed Abolghasem Ghadami¹, Francesco Bemporad¹, Fabrizio Chiti¹

¹ Dipartimento di Scienze Biomediche Sperimentali e Cliniche “Mario Serio”, Sezione di Biochimica, Università degli Studi di Firenze, Viale G. B. Morgagni 50, 50134 Firenze, Italy.
sghadami@unifi.it

INTRODUCTION: Transthyretin (TTR) is a homotetrameric protein that is one of 30 human proteins associated with systemic amyloidosis. TTR fibrillogenesis is accelerated by the presence of any of the approximately 100 different amyloidogenic mutations responsible for early-onset TTR amyloidosis. In spite of its link to human pathology, an anti-amyloidogenic effect that prevents fibril formation of the amyloid β (Aβ) peptide associated with Alzheimer’s disease (AD) has been proposed for TTR. In the light of the results obtained so far, one can hypothesize that TTR may act as an endogenous detoxifier of protein oligomers with potential pathological effects, to inhibiting amyloid fibril formation. In addition, previous data do not offer any insight into the mechanism by which TTR inhibits oligomer toxicity and on the TTR form responsible for such an effect. Furthermore, the key structural events inducing TTR to adopt an amyloidogenic conformation are not yet clear. In this study, I tried to address both aspects.

MATERIAL & METHODS: We labeled TTR with a coumarin derivative, which generates the FRET phenomenon with the endogenous tryptophan residues present in native TTR. We applied different techniques including X-ray fiber diffraction, optical absorption spectroscopy, far- and near-UV circular dichroism spectroscopy, intrinsic and extrinsic fluorescence spectroscopy, Fourier-transform- infra-red spectroscopy, stopped-flow devices, nuclear magnetic resonance spectroscopy, turbidimetry, dynamic and static light scattering, atomic force microscopy, and other biophysical and biochemical techniques. Finally, we examined the effects of TTR on the toxicity of extracellularly added oligomers formed by three different peptides/proteins (including Aβ), and thus the effects of the TTR-oligomer interaction.

RESULTS: DISCUSSION & CONCLUSIONS: DLS and ESI-MS show that DACM-labeled M-TTR is monomeric, fully labeled, and containing only one DACM moiety per protein molecule. We calculate the distances between Trp residues and the DACM by X-Ray Data. Unfolding and refolding traces were fit to multiexponential functions to determine the rate constants of unfolding and folding, together with their relative amplitudes. We obtained a Chevron plot for this experiment and showed that DACM-M-TTR is destabilized relative to unlabeled M-TTR due to increased unfolding rate. Additionally, other results showed that the labeled protein presented enhanced amyloidogenic propensity. Increasing of FRET efficiency would reflect decreasing of mean distance between two tryptophans and cysteine while aggregation occurs. Finally, although human transthyretin (TTR) is associated with systemic amyloidoses, an anti-amyloidogenic effect that prevents Aβ fibril formation in vitro has been observed. The ability of TTR to form soluble oligomers and insoluble fibrils, on the one hand, on to act as a molecular detoxifier, on the other hand, are based on the structural plasticity of the protein and thus on subtle conformational changes in the soluble state of TTR. FRET is an ideal technique to detect such subtle structural changes and is thus a very promising approach to address these two issues.

Figure 1 A the distances between Trp residues and the DACM calculated from X-Ray Data. The Figure B shows the FRET efficiency and the figure C shows the effects of TTR on the toxicity of oligomers.

Identification of genes that regulate light chain amyloidosis pathogenesis and clinical outcomes

H Landau, S Chung, BH Durham, S Devlin, H Hassoun, S Giralt, O Landgren, CY Park MD.

1Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY.
2Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY.

INTRODUCTION: Light chain (AL) amyloidosis is a plasma cell (PC) neoplasm characterized by the accumulation of misfolded light chains (LCs). Amyloidogenesis is due to the propensity of LCs to acquire mutations or assume a pathologic conformation, but cell-intrinsic characteristics of the pathologic PCs also likely contribute to aberrant protein/light-chain folding and have been poorly investigated. Thus, we have characterized transcriptomal and mutational changes in AL PCs. We also have correlated gene expression changes with clinical outcomes to identify novel prognostic biomarkers and modulators of response.

MATERIAL & METHODS: Pre-treatment PCs were purified from newly diagnosed AL patients (n=20) who underwent bortezomib and dexamethasone (BD) induction. Purified PCs were prepared, and RNA was extracted using the standard Trizol method. RNA-sequencing using Illumina HiSeq 2500 was used to generate 2x50 reads. Reads were aligned with STAR; RPKM value was calculated using an institutional r-make pipeline. Genome Analysis Toolkit was used for single-nucleotide variant and indel identification in the coding sequence with >8x coverage. Differential gene expression analysis, gene set enrichment analysis (GSEA), and mutational variant analyses were performed on the RNA-seq data. Common single nucleotide polymorphisms (SNPs), variants in pseudogenes and poorly characterized genes as determined from several databases were excluded. Remaining candidate mutations were evaluated using the Catalogue of Somatic Mutations in Cancer (COSMIC), and those with poor coverage were excluded. Remaining missense genetic variants were evaluated by several in silico analysis methods for predicted mutational effects on protein structure. Missense variants not predicted to be deleterious to protein structure were excluded, unless they were already documented in COSMIC or had a biological function relevant to the pathogenesis of AL. Surviving candidate missense and all appropriately covered/aligned disruptive (frameshift insertions or deletions, nonsense, and splice site) mutations were retained.

RESULTS: RNA-seq data were generated on pre-treatment PCs from 17 AL patients with an average sequencing depth of 177.31 million reads, despite the relative small number of PCs purified for these studies (range 1,470-392,983). Analysis of 6 CR/VGPR and 5 ≤PR patients identified 45 differentially expressed genes (threshold P value <0.0023, log2FC range 6.0 to -7.5). GSEA analysis of all differentially expressed transcripts reveals numerous pathways predicted to be up or downregulated, and genes differentially upregulated in CR/VGPR patients are those involved in apoptosis, ubiquitination and proteasome degradation, cell-mediated immunity, and cell-cell or cell-matrix interactions. In the 17 study cases, mutational candidates (n=52) were identified and included COSMIC-annotated mutations (25%; n=13/52), disruptive mutations (62%; n=32/52), in-frame insertions or deletions (indels) (10%; n=5/52), and other missense mutations (4%; n=2/52). The study cases had COSMIC-annotated mutations identified in the genes involved in apoptosis (53%; n=9/17), cell cycle regulation (29%; n=5/17), class I MHC-mediated antigen processing/presentation (18%; n=3/17), protein trafficking/transport (12%; n=2/17), transcriptional regulation (12%; n=2/17), tRNA processing (6%; n=1/17), and post-translational modification of nascent proteins (6%; n=1/17). Additionally, cases had disruptive mutations identified in genes associated with cellular functions important to protein stability (35%; n=6/17), the ubiquitin fusion degradation pathway (29%; n=5/17), and protein trafficking (24%; n=4/17).

DISCUSSION & CONCLUSIONS: This study performs both mutational and differential gene expression analysis on purified AL PCs. Our preliminary analyses demonstrate mutations in genes involved in cellular functions and pathways relevant to the pathogenesis of AL, provide insights into disease biology, and generate candidates for future functional genomic evaluation. Additional pre- and post-treatment samples, along with paired normal samples, will allow us to more rigorously evaluate the mutational landscape of AL amyloidosis, and correlate molecular features with clinical response or progression of disease. Primary data will be shared at the meeting.
Live cell imaging of IAPP and Aβ interaction

Ye Wang, Gunilla T. Westermark

Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden
Ye.Wang@mcb.uu.se

INTRODUCTION: Type 2 diabetes (T2D) and Alzheimer’s disease (AD) are chronic diseases, which at the same time are our most common forms of localized amyloidoses, islet amyloid polypeptide (IAPP) deposits in islets of Langerhans in association with T2D, and amyloid beta (Aβ) deposits in brain of patients with AD. Epidemiological studies show that individuals with T2D have a 50-60% increased risk of developing AD. Therefore, we hypothesize that IAPP-aggregation acts as seed for Aβ amyloid and that cross-seeding constitutes an underlying mechanism that links these two important diseases.

OBJECTIVE: To establish a cell-based assay for IAPP and Aβ aggregation and subsequent cellular effects using Bimolecular Fluorescence Complementation, BiFC.

MATERIALS & METHODS: Vectors with DNA corresponding to protein of interest – linker – part of fluorophore were generated and used for expression of IAPP-linker-YN173 (YFP 1-173); IAPP-linker-YC155 (YFP 155-239); YN173-linker-IAPP; Aβ-linker-YN173; Aβ-linker-YC155; YN173-linker-Aβ. The different combinations allow for studies on both parallel and anti-parallel interaction. HEK293 cells used for the study were seeded the day before transfection, and fluorescence was examined 24h after transfection using confocal microscopy, flow cytometry and transmission electron microscopy.

RESULTS: Specific interaction between IAPP and Aβ can be visualized using BiFC in living cells 24h after transfection. The interaction between IAPP and Aβ prefers parallel alignment of the peptides and expression of peptides in an anti-parallel fashion yielded a 50% reduction of fluorescence. Co-transfection of HEK293 cells with Aβ-linker-YN173 and Aβ-linker-YC155 resulted in the occurrence of distinct spots, indicative for amyloid formation within 24h after transfection.

Fig.1: Fluorescent HEK293 cells transfected with IAPP-linker-YN173 and IAPP-linker-YC155 (a), Aβ-linker-YN173 and Aβ-linker-YC155 (b) and IAPP-linker-YN173 and Aβ-linker-YC155 (c). In b, Aβ-expression results in the appearance of single or multiple dots in transfected cells different from the diffuse cytoplasmic fluorescence seen in a.
Tissue specific expression of TTR mutants in *Drosophila melanogaster*

Xiaohong Gu¹, Małgorzata Pokrzywa², Ingrid Dacklin⁴, Per Westermark², and Gunilla T Westermark¹

¹ Department of Medical Cell Biology, ² Department of Immunology, Genetics and Pathology, Uppsala University, SE-75123, Uppsala, Sweden. ³ Airoptic Sp. z o.o., 61-612, Poznan, Poland. ⁴ Department of Molecular Biology, Umeå University, SE-90187, Umeå, Sweden. xiaohong.gu@mcb.uu.se

**Background:** ATTR is known to be associated with senile systemic amyloidosis and hereditary forms of amyloidosis. The hereditary forms are linked to a great number of different mutations leading to various phenotypes, tissue distribution and age of onset. Amyloidogenic mutations in the TTR gene are known to destabilize the structure of the molecule, which leads to tetramer dissociation. This process results in the accumulation of misfolded TTR monomers that self-assemble into oligomers and then amyloid fibrils. We generated a *Drosophila* model of tissue specific expression to analyze if there is a tissue-specific association depending on mutation.

**Material and Methods:** The Gal4-UAS system was used to drive the expression of human wild type TTR and TTR with single mutations: TTR-L111M, TTR-L55P, TTR-A109S, TTR-V30L, TTR-A109T, TTR-R104H, and TTR-T119M to site specific expression in *Drosophila melanogaster*. We used Fatbody-Gal4 to express protein in the fat body, Hand-C-Gal4 to express in the heart and GMR-Gal4 in the eye.

**Results:** Western blot analysis of protein expression in wild type and transgenic flies showed that TTR monomers, dimers and tetramers were present. Protein expression results in accumulation of irregular-shaped aggregates composed of fibrillary structures mixed with highly ordered spherules under transmission electron microscopy.

**Conclusion:** Taken together these findings suggest that *Drosophila melanogaster* is a promising complementary system to study tissue specific pathology of TTR mutations.
Design of scaffold-stabilized amyloid inhibitors

C-L Towse, V Daggett

Department of Bioengineering, University of Washington, USA.

c towse@uw.edu

INTRODUCTION: A common feature of amyloidogenesis is the accumulation of insoluble protein fibrils following the formation of soluble oligomers. Evidence indicates that these soluble oligomers are the toxic species in amyloid disease and it has been hypothesized that they contain a form of structure, α-sheet, where the carbonyl groups and amide groups become orientated on opposing faces of the protein backbone (1). Heterochiral peptides containing L- and D-amino acids were rationally designed to form α-sheet structures and proposed to provide a complementary structure to interact with the α-sheet formed in amyloidogenic proteins (2). Although such peptides can inhibit amyloid formation, they are small (~20 residues) and dynamic. Our goal is to graft a successful inhibitory peptide design into a highly stable protein scaffold to increase the stability, activity and bioavailability of the inhibitor. Here, we have introduced peptide sequences into a segment of a well-characterized stable protein to produce structural chimeras and optimized designs using all-atom molecular dynamics (MD) simulations.

MATERIAL & METHODS: For each design, a segment of the wild-type protein scaffold was replaced with designed sequences using UCSF Chimera and dihedral angles from our heterochiral libraries (3). Each structure was then minimized and solvated by explicit, flexible F3C water molecules and simulated for 200 ns at 310 K as a microcanonical NVE ensemble using the in lucem molecular mechanics (ilmm) software package (4).

RESULTS: Anchoring peptide sequences with marginal inhibitory activity towards amyloidogenic proteins into a protein scaffold stabilizes the active conformation of the designed inhibitors. Reduction in the inhibitor sequence lengths can also produce inserts with little disruption to the stability of the protein fold.

DISCUSSION & CONCLUSIONS: Placement of designed inhibitors within a protein scaffold can produce desired stabilized inhibitory conformations. These designs are being optimized further in silico to produce different orientations of the designs within the protein scaffold to probe the structural specificity of the interactions between the inhibitor and amyloidogenic species to better understand the nature of the structural interaction with soluble oligomers.

REFERENCES:

Amplification of tissue-derived transthyretin amyloid

M M Rahman¹, B Schmuck¹, P Westermark², T Härd¹

¹ Department of Chemistry and Biotechnology, Swedish University of Agricultural Sciences, Uppsala, Sweden. ² Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden.

mahafuzur.rahman@slu.se

BACKGROUND:
Deposition of transthyretin (TTR) amyloid fibrils in body tissue results in TTR amyloid disease. Detection of TTR amyloid in tissue is critical for early diagnosis and effective treatment. The most utilized method for tissue amyloid detection is polarization microscopy after staining with Congo red. However, the observation using Congo red is limited by the size of particles that can be detected. The aim of this project is to extend the limit to even smaller particles, perhaps even before mature amyloid fibrils have developed.

METHODS:
Tissue amyloid was amplified by seeding with the TTR105-115-GGRADS fragment [1]. The induced fibrillation of TTR fragment was monitored by thioflavin T (ThT) fluorescence and fluorescent microscopy when biotinylated TTR fragment was used for seeding amyloid tissue sections.

RESULTS AND DISCUSSION:
The ThT kinetics profile of in vitro seeding shows that tissue-derived amyloid seeds the TTR fragment. A total of eleven tissue amyloid samples were tested, of which four came from patients affected with wild type TTR amyloidosis and seven from patients with mutant TTR amyloidosis. Some of the tissue samples share approximately the same fibrillation pattern and there are also examples of unique fibrillation profiles. Following this we did also explore seeded growth of TTR fragment in paraffin-embedded tissue sections. Amyloid in tissue sections were also amplified as expected. The biotinylated TTR was fibrillated on the section and produced fluorescence signals when streptavidin-coated Quantum dot was added to the tissue sample. Our preliminary result suggests that the project most likely will provide a new method for detection of very small amount of TTR amyloid fibrils deposited in tissue samples. We also hope that pre-fibrillar aggregates will be possible to detect.

REFERENCE:
The aggregation mechanism of amyloid β5-42

T Weiffert\textsuperscript{1}, S Linse\textsuperscript{2}

\textsuperscript{1}Department of Biochemistry and Structural Biology, Lund University, Lund, Sweden.
\textsuperscript{2}tanja.weiffert@biochemistry.lu.se

INTRODUCTION: In vivo, amyloid beta peptides (Aβ) exhibit great N-terminal and C-terminal heterogeneity. Inhibition of β-site amyloid precursor protein-cleaving enzyme 1 (BACE1), that normally cleaves on the N-terminal side when Aβ is generated, results in an increased amount of Aβ5-X though the total amount of Aβ decreases in cerebrospinal fluid.\textsuperscript{1} Hence we find it of great interest to investigate the aggregation mechanism of Aβ peptides starting at position 5 further.

MATERIAL AND METHODS: On the day of the experiment recombinant Aβ was dissolved in 6 M GuHCl and monomeric peptides were isolated by size exclusion chromatography. Aggregation kinetics for different Aβ5-42 concentrations was studied using ThT fluorescence measurements. Seeding and coaggregation experiments were performed in the same manner.

RESULTS: The aggregation propensity of Aβ5-42 was investigated by monitoring changes in ThT fluorescence over time. The aggregation data obtained over a range of initial monomer concentrations were fitted globally using the AmyloFit platform.\textsuperscript{2} The data was best fitted using a multistep secondary nucleation model with three free parameters: \( k_{n1}, k_{n2} \) and \( K_M \) (\( k_{n1} \) - primary nucleation, \( k_{n2} \) - elongation, \( k_{n2} \) - surface-catalyzed secondary nucleation, \( K_M \) - the Michaelis constant for saturation of secondary nucleation), figure 1. The combined rate constants are larger for Aβ5-42 than for Aβ42, hence aggregates Aβ5-42 faster than Aβ42.\textsuperscript{3} The ratio \( k_{n1}/k_{n2} \) describes the aggregate concentration above which secondary nucleation will produce more nuclei than primary nucleation and shows that secondary nucleation is significantly more important than primary nucleation for Aβ5- 42. The \( K_M \) value represents the monomer concentration when the secondary nucleation becomes half-saturated. Aβ42 and Aβ5-42 coaggregate together into fibrils and the two peptides also cross-seed. However, Aβ42 seems to be a better seed than Aβ5-42.

DISCUSSION & CONCLUSIONS: The N-terminally truncated Aβ5-42 lacks four amino acids compared to Aβ42, whereof two are negatively charged, resulting in a lower net charge of the Aβ5- 42 peptide. The observed deviations in the aggregation rate between full-length and truncated Aβ could be due to the lower net charge as well as the shorter length of Aβ5-42. Unlike Aβ42, where the secondary nucleation is ‘single-step’, is it the conversion to and detachment from the fibril of newly formed nucleus that is the rate determining step for Aβ5-42 aggregation within the studied concentration range.


Fig. 1: Aggregation kinetics data for 0.25-5 µM Aβ5-42. The results of global fitting to all data are shown with the curves at each concentration in the same color as the respective data points.
PA51

Structural and biochemical techniques reveal that charge interactions, polysaccharide geometry and fibril architecture play a crucial role in the mode of binding of glycosaminoglycans to amyloid-β fibrils

E Hughes¹, KL Stewart², EA Yates³, SE Radford² and DA Middleton¹

¹ Department of Chemistry, University of Lancaster, Lancaster LA1 4YB, United Kingdom. ² Astbury Centre for Structural Molecular Biology, School of Molecular and Cellular Biology, University of Leeds, Leeds LS2 9JT, United Kingdom. ³ Department of Biochemistry, Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, United Kingdom

e.hughes3@lancaster.ac.uk

The fibrillar amyloid-β (Aβ) deposits that accumulate in Alzheimer’s disease (AD) also contain polysaccharides of the glycosaminoglycan (GAG) class [1]. GAGs appear to associate preferentially with certain conformations of Aβ and can have an effect on the kinetics of self-assembly [2]. Targeting the aggregation pathway and reducing toxicity of Aβ peptides is a key therapeutic goal [3], and the influence GAGs have in both these areas makes them an important area of study. Here we use a number of select Aβ mutants in combination with GAG binding assays and magic angle spinning solid-state NMR (SSNMR), in an attempt to elucidate how these molecules recognise and bind to Aβ. We demonstrate that the GAG proxy heparin has a high affinity for Aβ40 fibrils with the 3Q conformation having three-fold cross-sectional symmetry [4]. Modifications to either Aβ or the GAG molecules alter the affinity of the interaction without changing the 3Q structure substantially. SSNMR results do not reveal any significant changes in Aβ conformation as a result of point mutations in the amyloid sequence, and both charged interactions and GAG geometry do appear to play a role in the mode of binding. We present a model of the heparin-Aβ fibril complex in which the binding site is located within a cleft formed at the junctions of the triangular structure. These data reveal that GAG-amyloid interactions display a range of affinities that critically depend on the details of the fibril architecture.

The effect of amyloid formation on the pKa values of α-synuclein

T Pálmadóttir¹, T Leiding¹, S Linse¹

¹ Department of Biochemistry and Structural Biology, Lund University, Sweden.
tinna.palmadottir@biochemistry.lu.se

INTRODUCTION: The formation of α-synuclein amyloid fibrils and their accumulation in inclusion bodies, termed Lewy bodies, are considered the hallmark of Parkinson’s disease. It has been found that the formation of α-synuclein fibrils are highly pH dependent, which is thought to be linked to the negatively charged C-terminal tail of α-synuclein [1]. The aim of this study is to examine the effect of fibrillation on the pKa values of the acidic residues in the C-terminal tail. The hypothesis is that the pKa values of the acidic residues in the C-terminal tail within fibrils are upshifted compared to monomers. The study also includes a development and evaluation of a method that can be used to investigate this matter.

MATERIAL & METHODS: The effect of amyloid fibril formation on the pH of α-synuclein in water was followed by measuring the pH of the sample before and after the fibril formation. The change in pH during fibril formation was also followed using the titration meter Probe Drum (Probation Labs, Sweden). Fibrils were examined using CD spectroscopy.

RESULTS: The pH of α-synuclein in water was measured before and after the formation of amyloid fibrils. A significant increase in pH was detected. The pH change was on average one pH unit (from 5.3-6.3) for samples in the concentration range of 25-80 µM. The number of protons taken up per α-synuclein molecule as it formed amyloid fibrils, was calculated to be in the range of 0.07-0.08 protons.

The effect of fibril formation on the pH of the sample was also examined by measuring the pH change simultaneously during the fibril formation (see fig. 1). The results were consistent with the previous ones: an increase in pH was observed during fibril formation.

DISCUSSION & CONCLUSIONS: An increase in pH during fibrillation indicates that protons are taken up as α-synuclein forms fibrils. This implies that α-synuclein fibrils have a higher affinity for protons, indicating an upshift in the pKa values of α-synuclein during fibrillation.

This is consistent with the hypothesis of the project: that the pKa values of the acidic residues in the C-terminal tail of α-synuclein fibrils are up-shifted compared to α-synuclein monomers. This upshift in pKa values could presumably be explained by a close proximity between negatively charged tails within fibrils. It should be noted that this pH change could also be related to a change in the pKa values of Histidine (His50), which has a pKa value of 6.78 in α-synuclein monomers [2].


Fig. 1: pH change during fibril formation of 35µM α-synuclein in water followed in the titration meter Probe Drum.
Infrared spectroscopic imaging: A label free approach for the detection of amyloidosis in human tissue biopsies

MJ Walsh¹, D Martinez¹, H Sreedhar¹, M Picken²

¹ Department of Pathology, University of Illinois at Chicago, Chicago, USA. ² Department of Pathology, Loyola University Medical Center, Maywood, USA.

INTRODUCTION: Detecting and sub-typing of amyloid deposits early in tissue biopsies is critical, especially with the emergence of new treatment strategies. Current techniques require suspicion of amyloidosis which then requires a tissue section being stained with Congo Red and examined using either polarized light or fluorescent light with a TRITC filter. Here we present a potential novel approach, Infrared (IR) Spectroscopic imaging, to identify abnormal protein deposits in a label-free approach based on the inerrant biochemistry of the tissue without the requirements for additional staining.

MATERIAL & METHODS: FFPE tissues (colon, pancreas and lung, n=10) were collected from the Loyola University Medical Center and sectioned at 4 microns on to special IR compatible slides. Serial sections stained with Congo Red were examined with a fluorescent microscope with a TRITC filter for the presence of amyloidosis and regions of interest identified. High resolution IR images were acquired and compared to the stained sections.

RESULTS: IR images collected from a range of tissues demonstrated difference in IR absorbance at certain biochemical frequencies, that could highlight regions of amyloidosis from unstained tissue sections. Furthermore, the amyloidosis was found to have a unique IR spectral signature compared to surrounding tissue.

DISCUSSION & CONCLUSIONS: This pilot study demonstrated that IR imaging represents a potentially useful approach to accurately identifying amyloidosis in tissues without requiring any stains to be used. Future work will focus on expanding the number of patients and the types of organs. We will also focus on determining whether we can subtype the amyloidosis class based on differences in the IR signature.
Diagnosis of hereditary ATTR amyloidosis using $^{11}$C-PIB-PET

N Ezawa$^1$, Y Sekijima$^{1,2,3}$, M Yazaki$^{1,2}$, K Oguchi$^1$, S-I Ikeda$^{1,2}$

$^1$Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan. $^2$Institute for Biomedical Sciences, Shinshu University, Matsumoto, Japan. $^3$Jisenkai Brain Imaging Research Center, Matsumoto, Japan.

nezawa@shinshu-u.ac.jp

INTRODUCTION:

Diagnosis of hereditary ATTR amyloidosis requires confirmation of amyloid deposition by tissue biopsy. However, tissue biopsy can evaluate only a limited area of the human body and specimen acquisition requires invasive procedures. On the other hand, Pittsburgh compound B (PIB)$^1$ has been used successfully in positron emission tomography (PET) studies to assess amyloid $\beta$ (A$\beta$) deposition in the brains of patients with Alzheimer’s disease$^2$. In addition, recent studies$^{3,4}$ indicated that PIB can be used to detect other types of amyloidosis. Here, we investigated the feasibility of using $^{11}$C-PIB-PET in diagnosis of hereditary ATTR amyloidosis.

MATERIAL & METHODS:

Subjects: Sixteen patients with hereditary ATTR amyloidosis (8 male, 8 female) with a mean age ($\pm$ SD) of 48.6 $\pm$ 10.9 years were enrolled in this study. The $TTR$ genotypes were as follows: V30M (p.V50M) heterozygous, n = 15; G47R (p.G67R) heterozygous, n = 1.

Methods: Thirteen patients underwent craniocervical PIB-PET and three patients underwent whole-body PIB-PET including the craniocervical region. For PIB-PET, subjects were positioned in the scanner 50 minutes after injection of 500 – 636 MBq of $^{11}$C-PIB, and scanned for 20 minutes in 3D-mode on a Discovery PET/CT 600 scanner (GE Healthcare, Milwaukee, WI).

RESULTS:

Increased $^{11}$C-PIB retention in the brain was found in 11 of 16 patients on craniocervical PIB-PET. PIB-PET also detected non-CNS ATTR amyloid deposition in the craniocervical region (i.e., amyloid deposition on scalp/muscle/connective tissue was found in 15 patients, lacrimal glands in 14 patients, parotid glands in 11 patients, and nasal mucosa in three patients). Whole-body PIB-PET revealed amyloid deposition in the heart (two of three patients) and kidney (one of three patients).

DISCUSSION & CONCLUSIONS:

The present study showed that $^{11}$C-PIB-PET can detect both CNS and non-CNS amyloid deposition in hereditary ATTR amyloidosis patients. These findings indicate that $^{11}$C-PIB-PET can be utilized in early diagnosis and systemic evaluation of amyloid distribution in ATTR amyloidosis patients. In addition, quantitative analysis of $^{11}$C-PIB-PET is expected to be useful in evaluation of therapy in such cases.

REFERENCES:

POSITRON EMISSION TOMOGRAPHY WITH 18F-FLORBETAPIR (FBP-PET) AS A DIAGNOSTIC TEST IN AMYLOIDOSIS

J. Mestre-Torres¹, C. Lorenzo-Bosquet², M. Gironella³, G Cuberas-Borrós², R. Solans-Laqué³, A. Fernández-Codina¹, S. Bujan-Rivas¹, J. Castell-Conesa³, F. Martínez-Valle¹

¹ Department of Internal Medicine, Vall d’Hebron Hospital, Barcelona, Spain. ² Department of Nuclear Medicine, ³ Department of Haematology, Vall d’Hebron Hospital, Barcelona, Spain.
fmartine@vhebron.net

INTRODUCTION: Amyloidosis comprises a group of diseases characterized by a deposition of amyloid fibrils which have an antiparallel B-sheet secondary structure. Research for evaluating evolution of amyloidotic deposits in patients under treatment must be done regularly with several tests. FBP is a radiotracer with tropism to proteins with β-sheet conformation that is used in Alzheimer’s disease.¹ The objective of the present study is to evaluate the utility of FBP-PET as a new diagnostic test in amyloidosis. To assess the utility of FBP tomography as a tool for studying the extension of amyloidotic deposits.

MATERIAL & METHODS: We performed an observational descriptive study with 13 subjects controlled at our Amyloidosis Unit: eleven patients with a biopsy-proven disease, one patient with senile amyloidosis and one woman who was healthy carrier of TTR mutation. A PET/CT was performed with injection of 370MBq FBP. Images were acquired after 40-50 minutes of injection and semi-quantitative description was done. Evaluation also included traditional techniques like echocardiogram, cardiac MRI, blood and urine tests, and electromyography. If other organs were involved, they were registered. The correlation between traditional techniques and PET/CT was studied.

RESULTS: The median age at diagnosis was 69 years (IQR: 60 – 75) and the median follow up 22.2 months (IQR 2 – 32). One patient had a hereditary amyloidosis by transthyrretin (TTR), 1 patient a senile amyloidosis, 8 patients a primary amyloidosis and 2 a secondary amyloidosis. One patient is considered to be a healthy carrier of mutated TTR protein. Traditional evaluation detected heart involvement in 8 patients (61.5 %), kidney involvement in 6 (46.15%) and soft tissue was involved in 4 patients (30.76%): 2 of them had macroglossia, 1 patient had thyroid gland enlargement due to amyloidosis and the remaining one had amyloid deposits in the stomach. Overall, excluding the patient who was a healthy carrier of TTR mutation, 11 out 12 patients had any kind of activity in FBP-PET. The patient without activity was diagnosed as having a senile amyloidosis by echocardiogram and cardiac MRI with a negative biopsy. FBP-PET showed cardiac involvement in seven patients (53.84%), kidney in 2 patients (15.38%) and tongue in 7 patients (53.84%). Other organs were involved: thyroid gland in six patients (46.15%), lung in five patients (38.46%) and spleen in 5 patients (38.46%). Multiple organs were involved in nine patients (69.23%). Correlation between traditional techniques and FBP-PET showed that four patients had both positive tests for heart involvement. Two patients identified as having cardiac involvement by traditional techniques did not have an increased uptake. On the other hand, four patients had a positive FBP-PET but could not be diagnosed with echocardiogram and/or cardiac MRI. Two of these patients had a left ventricular hypertrophy. Surprisingly, soft tissue involvement was higher than expected: two patients had macroglossia but 7 patients had an increased tongue uptake. Thyroid involvement was clinically suspected and confirmed in one patient with a goiter but had a positive FBP-PET in six. Lung and spleen involvement were not assessed as there was not a clinical suspicion prior to the FBP-PET findings.

DISCUSSION & CONCLUSIONS: FBP-PET is a promising technique that will help in amyloidosis diagnosis and for the evaluation of the burden of disease affected in patients with this disease.

Feasibility study of cardiac magnetic resonance elastography in cardiac amyloidosis

IC Chang\(^1\), A Arani\(^2\), SP Arunachalam\(^3\), M Grogan\(^1\), A Dispenzieri\(^3\), PA Araoz\(^{12}\)

\(^1\) Division of Cardiovascular Diseases, Mayo Clinic. \(^2\) Department of Radiology, Mayo Clinic.
\(^3\) Division of Hematology, Mayo Clinic, Rochester, Minnesota, USA.

Chang.Ian@mayo.edu

INTRODUCTION:

Magnetic resonance elastography (MRE) has been used clinically to assess tissue stiffness in diseases such as liver cirrhosis. However, whether MRE is feasible for myocardial stiffness is unclear. Cardiac amyloidosis “stiffens” the heart and leads to systolic and diastolic dysfunction. MRI with delayed enhancement is helpful in the diagnosis, but is often precluded due to frequently co-existing renal dysfunction. MRE is contrast-free and could potentially be a useful tool for diagnosis and treatment monitoring. The aim of this feasibility pilot study was to determine if MRE could differentiate the myocardial stiffness between a cardiac amyloidosis patient and healthy control.

MATERIAL & METHODS:

We identified endomyocardial-biopsy proven cardiac amyloidosis patients from the amyloid clinic at the Mayo Clinic. Age and gender matched controls were recruited from campus postings. Baseline clinical information was acquired from chart review. Subjects underwent a one-time MRE using a vibrating driver on the chest. Images were acquired with a 1.5T MRI scanner (Optima 450W, GE Medical Systems, Milwaukee, WI) at the minimum allowed delay after the R wave using a 3D cardiac-gated MRE spin echo pulse sequence, at a vibration frequency of 140Hz. Stiffness maps were generated using local frequency estimation inversion algorithm.

RESULTS:

One patient (58 years, male) and one control (57 years, male) were included in this pilot study. The patient was diagnosed with familial cardiac transthyretin amyloidosis with valine 122 isoleucine mutation. He had NYHA functional class II symptoms. Echocardiogram showed grade 3 diastolic dysfunction and reduced ejection fraction (EF) of 37%. The control had normal diastolic and systolic function. Short axis myocardial stiffness maps from the MREs are shown in Figure 1. The mean stiffness values were significantly different: 15.7 kPa and 8.8 kPa for the patient and control, respectively.

DISCUSSION & CONCLUSIONS:

Cardiac MRE was feasible in differentiating the myocardial stiffness between a cardiac amyloidosis patient and normal control. A larger study is needed to further establish the reference ranges of myocardial stiffness, and determine diagnostic and prognostic values of MRE in cardiac amyloidosis patients and controls.

REFERENCES:


Fig. 1: MRE stiffness maps of the patient with cardiac amyloid and normal subject
PA57

Cardiac uptake on $^{99m}$Tc-DPD scintigraphy as a novel marker of poor prognosis in systemic AL amyloidosis

J Zheng¹, D Hutt², J Gillmore², C Whelan², C Quarta², M Fontana², H Lachmann, S Mahmood, S Sachchithanantham, PN Hawkins² and A Wechalekar²

¹Department of Haematology, Royal Free Hospital, London, UK  ²National Amyloidosis Centre, University College London, London, UK

jiexin.zheng@nhs.net

INTRODUCTION:

Specific methods for imaging cardiac amyloid deposits and their utility in prognosis/monitoring have been limited. Echocardiograms have low sensitivity/specificity. Latterly cardiac magnetic resonance imaging appears to show promise. Bone scintigraphy tracers like technetium-99m-labelled 3,3-diphosphono-1,2-propanodicarboxylic acid ($^{99m}$Tc-DPD) have been extensively evaluated in transthyretin amyloidosis and now from the basis of a new non-invasive diagnostic algorithm. The utility and clinical importance of $^{99m}$Tc-DPD scintigraphy in AL amyloidosis has not been studied. We report here $^{99m}$Tc-DPD scintigraphy in a series of 189 patients with cardiac AL amyloidosis and the impact of cardiac $^{99m}$Tc-DPD uptake on outcomes.

MATERIAL & METHODS:

All patients who underwent $^{99m}$Tc-DPD scintigraphy, from June 2010-November 2015, with a confirmed diagnosis of AL amyloidosis with cardiac involvement were identified from the database of the UK National Amyloidosis Centre. All patients also had a full protocolized assessment for amyloidosis and were prospectively followed up. The characteristics of patients with cardiac uptake on $^{99m}$Tc-DPD scintigraphy were compared with patients without cardiac uptake. The survival outcomes were assessed by the method of Kaplan and Meier. Cox regression was used to determine univariate and multivariate variables impacting survival.

RESULTS:

A total of 189 patients were identified. One hundred and fourteen (60%) had no cardiac uptake on $^{99m}$Tc-DPD scan (DPD-negative) and 75 (40%) had cardiac uptake on $^{99m}$Tc-DPD scan (DPD-positive). The cardiac uptake was grade 1 in 57 (76%) of the patients, 13 (17%) had grade 2 and 5 (7%) grade 3 cardiac uptake. There was no significant difference between the DPD-positive and DPD-negative patients in terms of NYHA class I/II versus III/IV (64% and 36% in the DPD-positives and 67% and 33% in the DPD-negatives, p = 0.75); Mayo staging 1/2 vs. 3 (17% and 83% vs. 25% and 75%, p = 0.20); supine systolic blood pressure (112 vs. 116, p = 0.26), LVEF (50% vs. 55%, p = 0.1); mean LV wall (15mm vs. 15mm, p = 0.39) and e/e’ (18 vs. 17, p = 0.58). Non cardiac organ involvement did not show a significant difference between the DPD-positives and DPD-negatives (renal: 52% vs. 48%, p = 0.66; autonomic nervous system: 20% vs. 14%, p = 0.32). The serum free light chains showed no significant difference (median dFLC 343mg/L vs. 316mg/L, p = 0.40). However, the cardiac biomarkers were significantly higher in the DPD-positive patients compared to DPD-negative cases: N-terminal fragment of brain natriuretic peptide (NT-proBNP) (8296 ng/L vs. 4411 ng/L, p=0.005) and high sensitivity troponin (hsTNT) (113 ug/L vs. 83 ug/L, p=0.001) in patients with cardiac uptake on $^{99m}$Tc-DPD vs. those without cardiac uptake respectively. The median overall survival of the entire cohort was 7 months (95% CI 3 to 11 months). The median overall survival in DPD positive group was 4.3 months compared to 11.8 months in those without cardiac uptake on $^{99m}$Tc-DPD (p=0.045). On univariate analysis factors impacting survival were: cardiac involvement, Mayo disease stage, presenting dFLC, NT-proBNP, hsTNT and cardiac uptake on $^{99m}$Tc-DPD scintigraphy.

DISCUSSION & CONCLUSIONS:

$^{99m}$Tc-DPD scintigraphy shows cardiac uptake in 40% of patients with cardiac AL amyloidosis. Patients with cardiac uptake on $^{99m}$Tc-DPD scan have significantly higher NT-proBNP and hsTNT compared to patients with AL amyloidosis with cardiac involvement with no uptake on $^{99m}$Tc-DPD scan; even though the LV wall thickness is not significantly different. The outcomes of patients with cardiac uptake on $^{99m}$Tc-DPD scan are poorer than those with no cardiac uptake. The pathophysiology of $^{99m}$Tc-DPD uptake remains unknown. The poorer outcome and association with higher cardiac biomarkers suggests $^{99m}$Tc-DPD uptake is an indicator of greater myocardial damage. The technical simplicity, limited contraindications for scanning and low cost coupled with a marked prognostic impact suggests that $^{99m}$Tc-DPD scintigraphy could be considered as an additional simple baseline investigation in all patients with cardiac AL amyloidosis.
Myocardial uptake of $^{99m}$Tc-DPD in patients with AL amyloidosis

C de Miguel$^1$, L Llorente$^1$, FJ de Haro-del Moral$^2$, P García-Pavía$^3$, E González-López$^3$, J Segovia$^3$, I Krsnik$^1$

$^1$ Department of Hematology, Hospital Universitario Puerta de Hierro, Majadahonda, Spain.
$^2$ Department of Nuclear Medicine, Hospital Universitario Puerta de Hierro, Majadahonda, Spain.
$^3$ Department of Cardiology, Hospital Universitario Puerta de Hierro, Majadahonda, Spain.

Carlos.demiguel.j@gmail.com

INTRODUCTION: Amyloidosis is a rare disease in which differential diagnosis among the different subtypes is mandatory. A few cases have been described with deposition of 2 different proteins$^{1,2}$. We describe our experience with cardiac scintigraphy in the evaluation of patients with cardiac amyloidosis.

MATERIAL & METHODS: We have performed 493 cardiac scintigraphies with $^{99m}$Tc-DPD in the last 40 months in a single center. The indication was ventricular hypertrophy of unknown etiology. We have correlated the image findings with the clinical and/or pathological diagnosis.

RESULTS: Ninety cases (18.3%) showed $^{99m}$Tc-DPD uptake (positive scintigraphy). Eighty of these (92.2%) were diagnosed of TTR amyloidosis, whereas in 7 cases (7.8%) the diagnosis was AL amyloidosis. In 3 of these 7 patients (42.9%) the myocardial TTR amyloid deposition was demonstrated by immunohistochemistry techniques.

Among 47 patients diagnosed of AL amyloidosis, 7 (14.9%) showed a positive DPD uptake in cardiac scintigraphy. Six patients (85.7%) had AL amyloid deposits in the bone marrow. We have performed an endomyocardial biopsy in four of these patients (57.1%). In two of them (50%) we have demonstrated AL amyloid deposits and weak TTR amyloid deposition by immunohistochemistry techniques. One patient showed amyloid AL and TTR deposition by mass spectrometry. All patients showed endomyocardial DPD uptake. Five of them (71.4%) showed biventricular uptake and the other two univentricular. Furthermore, we observed DPD uptake in other locations in four patients (57.1%).

DISCUSSION & CONCLUSIONS: Cardiac $^{99m}$Tc-DPD scintigraphy has been described as a good method in the differential diagnosis of amyloidosis TTR vs. AL. However, in our experience, some cases of immunohistochemistry-proven AL amyloidosis show biventricular positivity. The actual incidence of deposit of both proteins (immunoglobulin light chains and TTR) is not known. In most centers mass spectrometry is not available as a routine. Mass spectrometry is recommended in cases with positive AL and TTR deposition by immunohistochemistry and positive $^{99m}$Tc-DPD uptake in cardiac scintigraphy.

REFERENCES:


Apical sparing left and right ventricular uptake of technetium pyrophosphate in transthyretin cardiac amyloidosis

BW Sperry1, MN Vranian1; A Tower-Rader1; R Hachamovitch1; M Hanna1, WA Jaber1

1Department of Cardiovascular Medicine, Cleveland Clinic Foundation, Cleveland Ohio

sperryb@ccf.org

INTRODUCTION: Nuclear scintigraphy using Technetium 99m-pyrophosphate (TcPYP) is used as a diagnostic tool in transthyretin cardiac amyloidosis (ATTR). Echocardiography has identified a pattern of decreased longitudinal strain in basal segments leading to a gradient of improved strain from basal to apical segments. The etiology of decreased basal segment longitudinal strain with relative sparing of the apex is unclear. We sought to examine the relative regional uptake of TcPYP in patients with ATTR.

MATERIALS & METHODS: We identified 35 consecutive patients with ATTR from 2011-2015 who underwent TcPYP scintigraphy. Attenuation correction single-photon emission computed tomography (SPECT) images were analyzed using 4DM software (Siemens) after manual image reformatting. Tracer uptake counts were determined for each of the 17 segments and average counts in the 6 basal, 6 mid, and 5 apical segments were calculated. Right ventricular uptake was scored in the basal, mid, and apical segments with a visual semiquantitative score (0=absent cardiac uptake, 1=mild uptake less than bone, 2=moderate uptake equal to bone, 3=high uptake greater than bone). Echocardiographic strain was assessed using velocity vector imaging.

RESULTS: In our cohort (age 79 ± 11 years, 69% male, 66% Caucasian, 34% hereditary ATTR), 13 deaths occurred over a median follow up of 295 days. TcPYP scintigraphy demonstrated increased LV uptake in basal and mid segments as compared to apical segments (Figure 1) which mimicked the apical sparing longitudinal strain pattern in echocardiography (Figure 2). RV uptake similarly was greater in the basal segments with 97% of patients having basal, 49% basal and mid, and 17% basal, mid and apical uptake.

DISCUSSION & CONCLUSION: There is a gradient of decreasing TcPYP uptake when progressing apically in the left ventricle, mimicking the corresponding improvement in regional echocardiographic strain. This pattern is also present in the right ventricle.
PA60

Quantitative $^{123}$I-SAP scintigraphy in the follow-up of AL amyloidosis patients

Ronald W.J. van Rheenen1, Bouke P.C. Hazenberg2, Rudi A.J.O. Dierckx1, Andor W.J.M. Glaudemans1

1 Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands
2 Department of Rheumatology & Clinical Immunology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands
r.van.rheenen@umcg.nl

INTRODUCTION: The clinical response and haematological response often do not coincide during treatment evaluation in AL amyloidosis patients. The $^{123}$I-SAP scintigraphy (SAP-scan) has potential to monitor changes in amyloid load of the body and individual organs. Visual grading of SAP scans only helps to detect large changes in the amyloid load. The aim of this study is to use quantitative $^{123}$I-SAP scintigraphy to detect smaller changes in amyloid load during follow-up of AL amyloidosis patients.

MATERIAL & METHODS: Between April 2009 and December 2015, 22 patients with AL amyloidosis underwent consecutive $^{123}$I-SAP scans with SPECT/CT (n=60) to evaluate the extent and distribution of the amyloidosis. In all patients AL amyloidosis had been biopsy-proven and typed. An earlier established ratio-based assessment enabled quantitative evaluation of all scans. These findings were compared to clinical parameters and the best haematological response, classified as complete response (CR), very good partial response (VGPR), partial response (PR), stable disease (SD), and progressive disease (PD).

RESULTS: All 22 patients had at least one scan after the baseline SAP-scan. Eleven patients had a second follow-up scan and five patients had a third follow-up scan. Best responses were: 7 CR, 5 VGPR, 5 PR, 4 SD and 1 indeterminate response. Often a slowly continuing decrease of SAP-binding was observed in cases with responsive disease and more than 1 follow-up SAP-scan (left Figure). Almost all complete responders showed quantitative improvement (right Figure). Some patients without defined organ disease could – by definition – not show an organ response, but they did show quantitative improvement of the SAP scan. When the quantitative values were compared to the clinical parameters a weak correlation was found between the liver ratio and Alkaline Phosphatase levels ($r = 0.31$, $p=0.028$) and between the kidney ratio and the eGFR ($r = -0.33$, $p=0.014$).

DISCUSSION & CONCLUSIONS: In this study a quantitative SAP-scan assessment was used to evaluate the treatment response in patients with AL amyloidosis. Complete haematological responders most clearly showed quantitative improvement. The current results need to be further evaluated in a larger follow-up cohort to study the variability of the quantitative results and the minimum change that is clinically relevant.

REFERENCES: none.
Coincidental amyloid deposition in a lung malignancy: not all $^{18}$F-FDG uptake is a sign of localized AL amyloidosis

R.W.J. van Rheenen, Bouke P.C. Hazenberg, Rudi A.J.O. Dierckx, Andor W.J.M. Glaudemans

1 Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

2 Department of Rheumatology & Clinical Immunology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

r.van.rheenen@umcg.nl

INTRODUCTION:

A $^{18}$F-FDG PET-scan (FDG-PET) can be a useful tool in the evaluation of localized AL amyloidosis. The report by Noordzij et al. demonstrated the additional value of FDG-PET to $^{123}$I-SAP scintigraphy (SAP-scan) regarding localized amyloid deposition in the lung. The aim of this report is to illustrate the coincidental presence of systemic amyloid in an unrelated malignant lesion of the lung.

MATERIAL & METHODS:

A 66-year-old female with biopsy-proven AL amyloidosis was referred to our hospital for the further evaluation of onset of polyneuropathy, nephrotic syndrome and a coincidental finding on a pulmonary CT-scan.

RESULTS:

The patient had a history of progressive sensory loss of her feet. Biochemical investigations showed an increased Alkaline Phosphatase of 621 U/l, an increased NT Pro-BNP of 1102 ng/l, proteinuria and a marginally increased Lambda Free Light Chain of 40 mg/l.

The SAP-scan showed a strongly increased SAP binding within the liver and spleen. Also it showed some SAP accumulation within the pulmonary mass in the right upper lobe. The FDG-PET showed increased FDG-uptake within the pulmonary mass, with signs of a necrotic centre. No other pathological FDG-uptake was reported elsewhere within the body.

It was concluded that due to the size and CT characteristics a malignancy was the most likely diagnosis and a lobectomy was performed. Afterwards the pathology report confirmed an adenocarcinoma with central deposition of amyloid instead of necrosis.

DISCUSSION & CONCLUSIONS:

Similar to the report of Noordzij et al. the pulmonary mass showed SAP accumulation and FDG-uptake, but due to the size, CT characteristics and the systemic nature of the AL amyloidosis other causes than localized AL amyloid deemed more likely. Whereas systemic AL amyloid in the lung tissue itself seemed to be absent, amyloid did accumulate in the malignant lung tumour as shown on the SAP-scan. We postulate that, in contrast to normal lung tissue, the changed microenvironment in malignant lung tissue favoured deposition of AL amyloid.

REFERENCES:

SPECT-based semi-quantitative assessment of $^{123}$I-SAP scintigraphy in patients with amyloidosis


1 Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

2 Department of Rheumatology & Clinical Immunology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

r.van.rheenen@umcg.nl

INTRODUCTION:

$^{123}$I-Serum Amyloid P (SAP)-scintigraphy is used to image the extent and distribution of amyloid deposition in patients with systemic AA, AL and ATTR amyloidosis in a non-invasive manner. The aim of this study was to develop a SPECT(-CT) organ/blood pool ratio-based quantitative assessment system with clear cut-off values to define organ involvement with high specificity. Some initial data has already been presented and this is a further account of ongoing work.

MATERIAL & METHODS:

Between January 2009 and January 2016 in 145 patients with amyloidosis a $^{123}$I-SAP with SPECT(-CT) was performed to evaluate the extent and distribution of the amyloidosis. In all patients amyloidosis had been proven and typed using a tissue biopsy. The patients were divided in five groups: AL type (n=86), AA type (n=20), ATTR type (n=18), AL localized type (n=21) and a disease-control group without amyloidosis (n=24). The latter was used to calculate the reference cut-off values for each organ ratio. The validity of these new values was tested by comparing ratio-based results to visual grading as well as to clinical parameters.

RESULTS:

Based on data of the control group the following cut-off ratios were calculated: liver 1.0, spleen 1.5, and kidney 1.1. When comparing ratio-based organ results to visual grading of the organs, normal values within the reference range were present in all amyloidosis patients visually graded 0. Values above the reference range were present in patients visually graded 2 or higher. Normal values, however, were often found in spleens and kidneys visually graded 1. When comparing organ values to the respective clinical parameters, modest correlations were found for liver values and Alkaline Phosphatase ($r = 0.33; p=0.028$), kidney values and eGFR ($r = -0.21; p=0.032$), and kidney values and proteinuria ($r = 0.45; p=0.04$).

DISCUSSION & CONCLUSIONS:

After increasing the number of patients of our earlier study it becomes more and more clear that a ratio-based assessment of SAP-scintigraphy enables us to obtain very specific, although less sensitive organ results of liver, spleen and kidney compared with our current visual assessment of these organs. Besides, the ratio-based method hints at a physiologically increased SAP binding within the spleen of controls. The amount of SAP binding in kidney and liver somewhat reflects clinical disease and probably the amyloid load of these organs. The latter may indicate utility of this quantitative method for monitoring patients with amyloidosis during the course of their disease and especially for assessing the effect of treatment.

REFERENCES: none
PA63

**DIAGNOSTIC VALUE OF ABDOMINAL FAT ASPIRATION IN CARDIAC AMYLOIDOSIS AND ITS CORRELATION WITH WHOLE BODY AMYLOID LOAD**

CC Quarta,1 E Gonzalez-Lopez,2 JA Gilbertson,1 N Botcher,1 T Youngstein,1 D Rowczenio,1 CJ Whelan,1 M Fontana, AD Wechalekar,1 HJ Lachmann,1 PN Hawkins,1 JD Gillmore.1

1National Amyloidosis Centre, Division of Medicine, UCL, Royal Free Hospital, London, UK.

2Heart Failure and Inherited Cardiac Diseases Unit, Department of Cardiology, Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain

c.quarta@ucl.ac.uk

**INTRODUCTION:** In suspected cardiac amyloidosis (CA), endomyocardial biopsy has a high diagnostic yield, but potential for serious complications including perforation underlies a reluctance to perform this procedure, especially in the elderly. By contrast, abdominal fat aspiration is a simple, safe and well-established procedure in systemic amyloidosis, but its diagnostic value in patients with CA remains unclear. We aimed to assess the diagnostic performance of abdominal fat aspiration to identify and type amyloid deposits in patients with aetiologically defined light chain (AL), hereditary transthyretin (ATTRm) or wild-type transthyretin (ATTRwt) cardiac amyloidosis.

**MATERIAL & METHODS:** We analysed the results of consecutive abdominal fat aspiration in patients diagnosed with CA at our centre between 2009 and 2015. Presence and type of CA was diagnosed as AL, ATTRm or ATTRwt based on a combination of serum FLC assay, IFE of serum and urine, genetic sequencing, endomyocardial and extra-cardiac biopsy, cardiac magnetic resonance (CMR), echocardiography, I123SAP and 99mTc-DPD scintigraphy. Abdominal fat tissue smears were stained with Congo red and immunohistochemistry (IHC) was performed on formalin fixed paraffin embedded tissue blocks using monospecific antibodies against known amyloid-forming proteins.

**RESULTS:** Fat aspirate samples from 642 patients with CA were analysed: 256 with AL (aged 65±10), 113 with ATTRm (aged 68±8; transthyretin (TTR) variants were distributed as follows: 69 V122I, 21 T60A, 7 V30M, 5 S77Y, 3 E89K, 2 F44L, 2 I68L, 2 I107F, 1 C10G, 1 E54L) and 271 with ATTRwt (aged 71±6). All patients had characteristic amyloid echocardiograms and/or CMRs, and all patients with either ATTRm or ATTRwt had Perugini grade 2 or 3 myocardial uptake of 99mTc-DPD. Among those with cardiac AL amyloidosis, 181/256 (71%) had amyloid on Congo red staining of their fat aspirate, and in 105/256 (41%) cases, the amyloid was successfully typed as AL by IHC. Amyloid was detected in the fat of 26/29 (90%) AL patients with a large whole body amyloid load by I123SAP scintigraphy, 33/39 (85%) patients with a moderate total amyloid burden and 120/188 (64%) with a small total amyloid burden (p=0.002). Amyloid was identified in 51/113 (45%) patients with ATTRm CA, and 37/113 (33%) had definitive IHC for TTR. Amyloid was identified in the fat samples of 23/69 (33%) patients with the most prevalent TTR variant, V122I, in contrast to 14/21 (67%) patients with T60A-associated CA. Among patients with ATTRwt CA, amyloid was identified in the fat of only 42/271 (15%) cases, and only 27/271 (10%) cases had diagnostic IHC for TTR.

**DISCUSSION & CONCLUSIONS:** This is the largest study so far exploring the diagnostic role of abdominal fat aspiration in patients with the three main aetiologies of CA. Abdominal fat aspiration was confirmed to have a high yield for the identification of amyloid deposits in AL, particularly in patients with extensive and widespread whole body amyloid. The diagnostic value of fat aspiration was substantially lower in ATTR amyloidosis, particularly ATTRwt and V122I-associated ATTRm, both of which have a predominant cardiac phenotype with limited extra-cardiac amyloid deposits.
Predictors of diphosphonates cardiac uptake in familial amyloidosis

M.S. Slama1, L. Eliahou1, H. Regaieg2, R. Chequer2, R. Ben Azzouna2, B. Mahida2, S. Dinanian1, V. Algalarrondo1, D. Adams1, D. Le Guludec2, F. Rouzet2

(1) Hopital Antoine Beclere, APHP, Cardiology Department, Universite Paris Sud, CRMR NNERF, Clamart, France (2) Hopital Bichat-Claude Bernard, APHP, Nuclear Medicine, Université Paris VII, Inserm U1148, Paris, France (3) Hopital Bicetre, APHP, Neurology, Universite Paris Sud, CRMR NNERF, Inserm U1195 , Le Kremlin-Bicetre, France

prmslama@gmail.com

INTRODUCTION: Cardiac amyloidosis is of major prognostic value in patients with mutated transthyretin (m-TTR) familial amyloidosis. Diphosphonate scintigraphy is an emerging diagnostic tool for m-TTR cardiac amyloidosis. We evaluated parameters likely to predict Diphosphonate cardiac uptake among these patients.

MATERIAL & METHODS: 155 consecutive patients with genetically and biopsy proven m-TTR amyloidosis or receivers of a liver transplant from a TTR-FAP donor (domino) were prospectively included. Demographical (age, sex) and biological (Troponin, BNP value) data, mutation type, history of (domino) liver transplantation or Tafamidis therapy and echocardiography results (LVEF, interventricular septum (IVS) thickness, global LV strain) were recorded. Patients were randomly injected with DPD or HMDP, followed by chest SPECT 3 hours later. Myocardial uptake was considered positive when the ratio between 3D isocount volume of interest (VOI) generated over the myocardium and a standard VOI in the right lung (H/L) was >2 as previously determined.

RESULTS: Seventy patients presented a positive cardiac uptake and 85 did not. On univariate analysis, the determinants of a positive uptake were age (69±9 vs. 50±13,p<0.0001), IVS thickness (16±17 mm vs. 11±8 mm, p<0.0001), reduction of the LVEF (59±12 vs. 65±8, p=0.001), BNP value (226±346 vs. 72±121, p=0.001), but not DPD as a tracer vs HMDP (64% vs. 51%, p=0.1) male gender (67% vs. 54%, p=0.1), Troponin value (0.069±0.058 vs. 0.050±0.044, p=0.06), strain (-15,8 vs. -15,4, p=0.7), or Val30Met mutation (51% vs. 51%, p=1) whereas history of liver transplantation was a determinant of absence of uptake (21% vs. 39%, p=0.02). Multivariate analysis using stepwise logistic regression showed that independent determinants of cardiac uptake were patient age (p<0.0001; odds ratio (OR)=1.13; 95% confidence interval (CI): 1.07 to 1.20) IVS thickness (p<0.0001; OR=1.4; 95% CI: 1.2 to 1.7) and the presence of domino liver transplant (p=0.003; OR=0.13; 95% CI: 0.03 to 0.50).

CONCLUSIONS: The present study shows that in patients with m-TTR amyloidosis, IVS thickness and patient age are independent predictors of diphosphonate cardiac uptake, whereas domino liver transplantation was associated with a lower risk. These results suggest that the exposure duration to mutated TTR is a key parameter determining diphosphonate cardiac uptake.
Cardiac denervation occurs before amyloid deposition in mutated TTR amyloidosis: a scintigraphic study

INTRODUCTION: In familial mutated TTR amyloidosis, cardiac involvement is of major prognostic value. In this setting, two approaches based on radionuclide imaging proved relevant: assessment of sympathetic denervation with $^{123}$MIBG and detection of amyloid deposits with diphosphonates imaging (DPD). We compared the results of both modalities in aTTR patients with suspected cardiac involvement.

MATERIAL & METHODS: 76 consecutive patients with genetically and biopsy proven mutated m-TTR amyloidosis were prospectively included (males 62%, Val30Met: 47%, domino-liver transplantation 13%, symptomatic: 57%, interventricular septum (IVS) ≥12 mm: 52%; left ventricular ejection fraction: 63±10%). All underwent both $^{123}$MIBG and DPD scintigraphy with a delay <3 months. $^{123}$MIBG heart-to-mediastinum ratio (HMR) was calculated on planar acquisitions performed 4 h after tracer injection. Cardiac $^{123}$MIBG was scored as normal (HMR≥1.9), mildly (1.9>HMR≥1.6), moderately (1.6>HMR≥1.3), or severely (HMR<1.3) abnormal. DPD SPECT acquisitions were performed 3 hours after tracer injection. Cardiac uptake was visually scored as present or absent and quantified by the ratio between 3D isocount volume of interest generated over the myocardium and a standard volume in lung (H/L).

RESULTS: The delay between DPD and $^{123}$MIBG was 6±12 days. DPD showed cardiac uptake in 30 patients (39%) and $^{123}$MIBG HMR was < 1.6 in 50 patients (66%; p=0.002). When $^{123}$MIBG was normal (n=26), DPD was normal except for 1 patient. When $^{123}$MIBG was abnormal (n=50), DPD was normal in 21 patients (42%). The uptake of DPD increased with the denervation score (normal: 0.6±0.2; mild: 0.6±0.4, moderate: 3.4±3.3; severe: 4.5±3.7; p<0.001 between normal and moderate/severe). In patients with a previous domino liver transplantation (n=10), the overall pattern was similar. In asymptomatic patients (n=31), $^{123}$MIBG was abnormal in 45% (n=14), among whom 50% had a normal DPD; all those with a normal $^{123}$MIBG (n=17) had a normal DPD. Compared to symptomatic patients, $^{123}$MIBG HMR was greater (1.8±0.3 vs. 1.6±0.4; p=0.008) and DPD H/L was lower (1.7±2.3 vs. 3.2±3.5; p=0.04).

CONCLUSIONS: in patients with suspected cardiac involvement of m-TTR amyloidosis, $^{123}$MIBG was abnormal earlier and more frequently than DPD. Among asymptomatic patients, $^{123}$MIBG was abnormal in 45% of patients, and only half of those with cardiac denervation had a positive DPD. This suggests that cardiac denervation is more frequent and occurs earlier than significant cardiac amyloid deposits in aTTR- amyloidosis.
Cardiac output is a useful prognostic echocardiographic marker in patients with wild-type transthyretin amyloidosis.

P Milani1,2, A Dispenzieri1, MA Gertz1, RA Kyle1, G Lin3, KW Klarich1, G Merlini2, WL Miller3, AL Clavell3, DD Borgeson3, BL Karon3, BA Boilson3, M Grogan3.

1Division of Hematology, Mayo Clinic, Rochester, MN, USA. 2Amyloidosis Research and Treatment Center, Foundation IRCCS Policlinico San Matteo, and Department of Molecular Medicine, University of Pavia, Pavia, Italy. 3 Division of Cardiovascular Disease, Mayo Clinic, Rochester, MN, USA.

INTRODUCTION: Cardiac wild-type transthyretin (ATTRwt) amyloidosis is an underappreciated cause of death in the aging population. The diagnosis is now based on echocardiography, histologic confirmation, and bone scintigraphy. The aim of this study is to identify echocardiographic prognostic markers in a large ATTRwt cohort.

MATERIAL & METHODS: Patients seen between March 1984 and December 2013 were eligible for this retrospective study if they had an echocardiogram at the Mayo Clinic, Rochester, MN within 6 months of their ATTRwt diagnosis. All patients had measurements of left ventricular chamber size and wall thickness, stroke volume (SV) and ejection fraction (EF). Patients with the diagnosis later that 2003 (143 subjects) also had measurement of cardiac biomarkers --N-terminal fragment of the brain natriuretic peptide type B (NT-proBNP), cardiac troponin (cTnT)-- in addition to the baseline laboratory data. Thresholds of continuous variables were chosen based on receiver operator curves targeting death at 1-year. Cox proportional hazards analysis was used to identify factors that were prognostic for overall survival (OS). Statistical analyses were done using JMP 10.0 (SAS, Cary, NC).

RESULTS: Among the 300 patients satisfying entry criteria, median age was 75 years (range 53-92) and 91% were male. All patients had biopsy proven diagnosis of ATTRwt, 229 (78%) had a positive endomyocardial biopsy, the remaining patients had typical cardiac imaging findings with a positive extracardiac biopsy. Median echocardiographic baseline variables were left ventricular thickness of 17 [interquartile range (IQR): 15-19.5], left ventricular mass indexed/body surface area 161 g/m² (IQR: 132-191), cardiac index (CI) 2.42 L/min/m² (IQR: 2.03-2.87), cardiac output (CO) 4.68 L/min (IQR: 3.99-5.69 L/min). The median NT-proBNP was 2902 ng/L (IQR: 1649-6264 ng/L), troponin T 0.04 ng/mL (IQR: 0.02-0.08 ng/mL). Median overall survival (OS) from diagnosis was 3.4 years [confidence interval (CI): 3.05-3.74 years]. On univariate analysis the baseline echocardiographic variables predicting OS were EF ≤50% [relative risk (RR): 1.67, P=0.0004], SV-index ≤33 mL/m² (RR 1.74, P=0.0009), cardiac index ≤2.26 L/min/m² (RR 1.68, P=0.001) and CO ≤3.8 L/min (RR 1.86, P=0.001). Other clinical variables predicting OS were Mayo ATTRwt stage III (NT-proBNP ≥3000 ng/L and cTnT ≥0.05 ng/mL; Grogan et al.). In separate multivariate models adjusted for age and EF, SVI ≤33 mL/m² (RR 1.5, P=0.01), AND CO ≤3.8 L/min (RR 1.7, P=0.004) were independent prognostic factors. Only CO and ATTRwt-stage III retained significance when cardiac biomarker stage was incorporated into the model (Table).

DISCUSSION & CONCLUSIONS: CO resulted as independent predictor of survival in an unselected cohort of patients with ATTRwt. This variable is part of the routine ECHO evaluation, is easy to calculate and adds prognostic information in addition to cardiac biomarkers.

Table. Multivariate model according to echocardiographic variables and cardiac biomarkers staging.

<table>
<thead>
<tr>
<th>Variable</th>
<th>RR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.09 (1.05, 1.14)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EF ≤50%</td>
<td>1.57 (0.92, 2.70)</td>
<td>0.09</td>
</tr>
<tr>
<td>CO ≤3.8 L/min</td>
<td>2.14 (1.20, 3.72)</td>
<td>0.01</td>
</tr>
<tr>
<td>ATTRwt-stage III</td>
<td>3.41 (1.89, 6.04)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Nano-scale infrared imaging of β-sheet structures in synaptic junctions of primary neurons isolated from transgenic mice, models of Alzheimer’s disease

O Klementieva¹, A Engdahl², P Uvdal²,³, LM Miller⁴, G Gouras¹

¹ Department of Experimental Medical Science, Lund University, Lund, Sweden. ² MAX IV Laboratory, Lund University, Lund, Sweden. ³ Chemical Physics, Department of Chemistry, Lund University, Lund, Sweden. ⁴ National Synchrotron Light Source, Brookhaven National Laboratory, Upton, USA

Oxana.Klementieva@med.lu.se

INTRODUCTION:

Amyloid β is a class of aggregation-prone proteins, which may misfold into stable, β-sheet rich fibrils. Amyloid β is linked to the development of synaptic pathology in Alzheimer’s disease (AD)¹. However, a main question in the AD field is how amyloid β contributes to AD neuropathology? Up to now there is little evidence for β-sheet formation at the sub-cellular level in neurons. Our aim was to study β-sheet structures in synaptic junctions of AD transgenic neurons. We aim to understand the mechanism by which amyloid β is involved in synaptic pathology in AD.

MATERIAL & METHODS:

To study β-sheet structures at a sub-cellular level in AD neurons (APP/PS1) we used a new technique which combines scattering-scanning near-field optical microscopy and mid-infrared synchrotron radiation (IR s-SNOM). Scanning in nanometer proximity to the sample with the tip of an atomic force microscope (AFM) this new approach enables molecular vibrational spectroscopic imaging with nano-scale spatial resolution (~ 40 nm) in the full mid-infrared (1000-5000 cm⁻¹) region. Moreover, synchrotron infrared spectroscopy imaging is a direct method to target specifically β-sheet structures in their native state since no sample processing, e.g. purification/concentration procedures or labelling with conformation specific antibodies nor dyes are required. In this way chemical structures that could be affected or lost during chemical processing remain in situ and contribute to the infrared spectrum²,³. IR s-SNOM experiments were done at Advanced Light Source, Lawrence Berkeley National Laboratory, USA.

RESULTS:

Using synchrotron-based infrared micro-spectroscopy imaging (Maxlab, Lund, Sweden and NSLS, Brookhaven, USA) we studied the secondary structure of proteins in cultured neurons at the micro level. The analysis of protein secondary structure showed a significantly higher ratio of β-sheet in AD transgenic cultured neurons expressing AD mutant APP compared to wild-type neurons, suggesting that the abnormal (β-sheet rich) protein structure occurs within AD neurons. Here we report that using IR s-SNOM we imaged neurons at nanometer scale (neuronal synapses). Moreover, analyzing Amide I peak positions, which appeared to be shifted in AD transgenic neurons, we obtained structural information about β-sheet structures in these synaptic junctions.

DISCUSSION & CONCLUSIONS:

Our results show that β-sheet formation can initiate within AD transgenic neurons and their synapses. However, since identifying the neurotoxic agents is a top priority in the AD field, further experiments are required to to understand how β-sheet rich variants of proteins may propagate from one neuron to the next, seeding misfolding and triggering aggregation on the way, and nano-scale infrared imaging could a useful tool in this study.

REFERENCES:

Clinical and $^{123}$I-SAP scintigraphy findings in three members from a family affected by AGel amyloidosis

RW.J. van Rheenen$^1$, Bouke P.C. Hazenberg$^2$, Rudi A.J.O. Dierckx$^1$, Andor W.J.M. Glaudemans$^1$

$^1$ Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

$^2$ Department of Rheumatology & Clinical Immunology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

r.van.rheenen@umcg.nl

INTRODUCTION:

An earlier study by Rowczenio et al. discussed the clinical characteristics and $^{123}$I-SAP scintigraphy (SAP-scan) in 10 cases with gelsolin-derived (AGel) amyloidosis (1). With this report we aim to increase the caseload to 13 patients.

MATERIAL & METHODS:

Within the Dutch amyloidosis patient population there are three family members (father, son and daughter) with AGel D187N Amyloidosis. In February 2016 their regular check-up included an added SAP-scan.

RESULTS:

The father, born in 1934, was 50 years old when AGel amyloidosis was detected in his eyes (cornea lattice dystrophy), a disease already known to be present in his father and aunt. Later the mutation was characterized as D187N. He developed signs of cutis laxa later and needed facial surgery. He developed signs of polyneuropathy at 79 years and at that time for the first time also some loss of renal function (eGFR 50) was detected without proteinuria. His daughter, born in 1960, also had proven amyloid deposition and the same mutation. She only had some dryness of the eyes, facial weakness around the eyes and she had a normal renal function. His son, born in 1963, also had proven amyloid deposition and the same mutation. He needed facial surgery for his eyelids when he was 42, had a normal kidney function and little proteinuria.

All three patients showed clearly increased SAP binding within the kidneys, even though only the father had clinical signs of kidney involvement. The spleen visually seemed to show some uptake in all three, but quantitatively no increased uptake was present in the spleen, liver or elsewhere in the body.

DISCUSSION & CONCLUSIONS:

When compared to the already described 10 AGel cases these 3 family members seem to seamlessly fall into the fold. As remarked in the earlier study, also in two of the three cases there was kidney involvement on the SAP-scan without matching clinical features. The oldest of the three did not show clear loss of kidney function until he was almost 80 years old.

REFERENCES:

Activation of TTR amyloid clearance with conformation-specific, human-derived monoclonal antibodies against misfolded TTR

A Michalon, B Combaluzier, E Varela, C Huy, A Hagenbuch, OB Suhr, MJ Saraiva, J Grimm

1Neurimmune, Zürich, Switzerland; 2Department of Medicine, Umeå University Hospital, Umeå, Sweden; 3IIS, Instituto de Investigación e Innovação em Saúde, University of Porto, Porto, Portugal

aubin.michalon@neurimmune.com

The formation of transthyretin amyloid (ATTR) is a spontaneous process driven by thermodynamics and occurs at low rate under physiological conditions. It is initiated by the dissociation of TTR tetramers into monomers, which then adopt the energetically more stable amyloid conformation. For reasons which are not well understood, this process may accelerate and overcome the natural resorption capacity of the body, leading to ATTR amyloidosis in patients who may or may not be carriers of TTR gene mutations. Polyneuropathy and/or cardiomyopathy with heart failure dominate the clinical presentation of the disease.

Like other amyloid diseases, the presence of antibodies directed against ATTR was observed in FAP patients and shown to correlate with later disease onset, indicating that the immune system can participate in the elimination of misfolded TTR proteins and confer protection against the disease process.

To exploit this mechanism for therapeutic purposes, we screened the immune repertoires of FAP patients, presymptomatic mutation carriers and healthy controls, and generated a set of recombinant human-derived monoclonal antibodies which bind with high affinity and selectivity to ATTR but not physiological TTR. These antibodies recognize WT-TTR amyloid deposits in cardiac tissue from patients with cardiomyopathy, and V30M-TTR amyloid deposits in biopsies from FAP patients. In vitro, low nanomolar concentrations of antibody were sufficient to activate dose-dependent phagocytosis of soluble misfolded TTR oligomers. In ex-vivo studies on tissues from cardiomyopathy patients, selected antibodies triggered the recruitment of macrophages to ATTR plaques and phagocytic clearance resulting in a reduction in amyloid plaque number and intensity.

These human-derived monoclonal ATTR antibodies are promising candidates for the development of disease-modifying therapies targeting immune-mediated clearance of TTR aggregates.
The role of gender and onset age as predictors of ophthalmologic changes in transthyretin familial amyloid polyneuropathy (TTR-FAP) patients

Natália Ferreira¹, Ana Carolina Abreu¹, Inês Carneiro², Isabel Fonseca², Maria do Carmo Vilas-Boas², Teresa Coelho².

¹ Unidade Corino de Andrade, ²Centro Hospitalar do Porto, Porto, Portugal

Introduction
To evaluate predictors of specific ocular changes in TTR-FAP patients on tafamidis

Methods
We performed a retrospective analysis of 129 patients transthyretin familial amyloid polyneuropathy (TTR-FAP) disease, on tafamidis, to study the influence of gender, the onset age, as well as the evolution time of the disease (years) on the occurrence of several ophthalmological events (amyloid deposits on lens anterior capsule, amyloid deposits on pupillary border, scalloped pupil, and vitreous opacities). The analyses were done using proportional hazards Cox multivariable regression models. The follow-up time was calculated since the first ophthalmologic evaluation until February, 2016.

Results
Sixty-eight patients were female (53%). Mean onset age was $39 \pm 13$ years, the mean evolution time of the disease was $1.8 \pm 9$ years at the beginning of tafamidis therapy, and the mean follow-up time at first ophthalmologic evaluation was $4.8 \pm 3.3$ years. At the end of the study (February, 2016), amyloid deposits on anterior lens capsule were present in 27 right eyes and in 22 left eyes, amyloid deposits on pupillary border was present in 21 and 18 right and left eyes and scalloped pupil in 8 right and 11 left eyes. Amyloid vitreous opacities were present in 36 right and left eyes.

Mean onset age was significantly higher in female gender ($46 \pm 12$ vs. $36 \pm 13$ years, $P=0.011$). No significant changes were found in gender in relation to mean evolution time of the disease at the first time of evolution.

Female gender was significantly associated with higher risk (higher hazard ratio) of amyloid deposits on lens anterior capsule, amyloid deposits on pupillary border, and vitreous opacities, independently of the onset age and the evolution time of the disease. The higher mean onset age was also statistically significant associated with a higher risk of scalloped pupil, amyloid deposits on anterior lens capsule, amyloid deposits on pupillary border, and vitreous opacities. No significant association was found between years of evolution of the disease and the ophthalmologic events. The interaction between gender and onset age was also tested but it was removed from all model due to non-significance.

Conclusions
Female gender and higher onset age are independent predictors of ophthalmological changes, namely amyloid deposits on lens anterior capsule, amyloid deposits on pupillary border, scalloped pupil, and vitreous opacities.
Haplotype analysis of the transthyretin V122I allele associated with cardiac amyloidosis (rs76992529): implications for origin(s) of the variant allele

DR Jacobson¹, AA Alexander²

¹ Medical Service, Veterans Affairs Boston Healthcare System, Department of Medicine, Boston University School of Medicine, and Amyloidosis Center, Boston University School of Medicine/Boston Medical Center, Boston, MA, USA.
² Research Service, Veterans Affairs Boston Healthcare System, Boston, MA, USA. daniel.jacobson@va.gov

INTRODUCTION: The common transthyretin (TTR) variants G6S, V30M, T119M, and V122I each arose from a G to A transition at a CG dinucleotide, a mutational “hot spot,” increasing the likelihood of multiple independent origins for these variants. Several studies have used single nucleotide polymorphisms (snps) in the introns and nearby flanking regions of the TTR gene to investigate the origin(s) of the TTR V30M variant (p.V50M) but to date, no similar studies of the other common TTR variants have been published. TTR V122I (p.V142I) is associated with cardiac amyloidosis over age 60.¹ The variant is carried by 3.4% of African Americans² and by over 5% of the population in a contiguous area of Africa ranging from Guinea in the west to Burkina Faso and Ghana in the east, with a lower prevalence outside those countries (submitted for publication). These data are consistent with a single founder, but do not rule out the possibility of a few founders in that part of Africa. Haplotype analysis, which can enable further investigation of the allele’s origin, typically relies on studies of multiple relatives within each kindred. In the absence of pedigree information, haplotype analysis can be performed using allele-specific PCR if informative snps are within a few kb of each other, or limiting-dilution PCR for longer distances.

MATERIAL & METHODS: We have used two snps in TTR intron 3 (rs7235277 [G/C] and rs1791227 [T/C], both within 2 kb of codon 122) to perform haplotype analysis on DNA samples from 173 African-Americans (six V122I homozygotes, 107 V122I heterozygotes, and 60 samples negative for the variant allele). Samples were first genotyped by PCR and restriction digestion using enzymes Fnu4HI and SacI. For samples where genotyping did not permit haplotype assignment, allele-specific PCR of the V122I allele was performed followed by digestion of the PCR product derived from the variant allele.

RESULTS: Both snps were found to be polymorphic for the two expected alleles in the control (i.e. V122I-negative) African-American population, at comparable frequencies to the expected frequencies from the online dbsnp database. The normal (V122) allele was found on all four possible haplotype backgrounds defined by these two snps. In contrast, the V122I variant allele was polymorphic at only the snp farther from codon 122, i.e., it was found on only two haplotype backgrounds (table 1).

DISCUSSION & CONCLUSIONS: It is notable that both sequences at the snp 1.7 kilobases from codon 122 were found in similar frequencies on the normal and V122I allele, whereas all of the V122I alleles contained the identical sequence at the closer snp (0.8 kb from codon 122). These data are consistent with a single ancient V122I founder on haplotype I or III, followed by a crossing over between the two intronic snps and evolutionarily neutral spread of the variant. Alternatively, these data do not rule out the possibility of two founders, one each on haplotype backgrounds I and III; however, the simplest hypothesis consistent with these haplotype data and data on the prevalence of the variant in several African countries is that the vast majority of modern-day TTR V122I carriers are descended from a single ancient founder.


Table 1. Haplotype backgrounds of the normal and V122I alleles.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>V122 (normal) allele</th>
<th>V122I (variant) allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (G,T)</td>
<td>133</td>
<td>80</td>
</tr>
<tr>
<td>II (G,C)</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>III (C,T)</td>
<td>62</td>
<td>39</td>
</tr>
<tr>
<td>IV (C,C)</td>
<td>9</td>
<td>0</td>
</tr>
</tbody>
</table>

Funding: The study was supported by Merit Review funds from the Department of Veterans Affairs (DRJ).
Myocardial contraction fraction stratifies prognosis in cardiac AL and m-ATTR cardiac amyloidosis

S. Perlini1,2, P. Milani2, F. Salinaro1, R. Mussinelli1, F. Musca1, G. Rizzola1, G. Gioia1, L. Obici2, G. Palladini2, G. Merlini2

1Clinica Medica 2, Dept. Internal Medicine, and 2Amyloidosis Research and Treatment Center, Dept. Molecular Medicine, Foundation IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy

stefano.perlini@unipv.it

INTRODUCTION: In cardiac amyloidosis ejection fraction (EF) is often preserved until the late stage of the disease in the vast majority of patients, who therefore fulfil the definition of heart failure with preserved ejection fraction (HFpEF). However, many indices of systolic function – such as midwall fractional shortening (MWFS), mitral annulus longitudinal systolic excursion (MAPSE), and tissue Doppler systolic peak (TDI-S2) - are depressed, showing evidence of systolic impairment despite preserved ejection fraction. A relatively new index of chamber systolic function is the myocardial contraction fraction (MCF), a volumetric measure of myocardial shortening that is defined as the ratio between stroke volume (SV) and myocardial volume (MV, i.e. left ventricular mass divided by the 1.05 g/ml, the mean density of myocardium).

MATERIAL & METHODS: To compare MCF with other indices of systolic function, i.e. EF, MWFS, MAPSE, and TDI-S2 in different aetiologies of cardiac amyloidosis, 173 cardiac light-chain (AL) and 102 mutated transthyretin (m-ATTR) amyloidosis patients underwent two-dimensional echocardiography at diagnosis. Cox proportional hazard modeling was used to determine the association of the different systolic indices with survival, over a median follow-up of 38.9 months (range, 19-75 months).

RESULTS: When comparing m-TTR with AL cardiac amyloidosis, no difference was observed in EF (57±9 vs. 58±10 %; p=ns), MAPSE (9.3±3.4 vs. 8.6±3.4 mm, p=ns), and TDI-S2 (9.0±3.6 vs. 8.6±3.5 mm, p=ns). In contrast, both MWFS (10.3±1.4 vs. 11.2±1.5%; p<0.05) and MCF (21.9±9.8 vs. 28.5±11.1; p<0.001) were lower in cardiac m-ATTR, indicating a higher degree of systolic impairment in the mutated transthyretin-related when compared with the light-chain cardiac amyloidosis aetiologies. In cardiac AL, MWFS% and MCF – but not EF – resulted independent predictors of overall survival. In contrast, in cardiac m-ATTR amyloidosis the only independent prognostic factor was MCF, whereas the other indices of systolic function did not enter the model.

DISCUSSION & CONCLUSIONS: Myocardial contraction fraction is superior to ejection fraction in predicting overall survival among HFpEF patients caused by either AL or m-ATTR cardiac amyloidosis.
Senile systemic amyloidosis and osteoarthritis: expanding the disease spectrum in humans and transgenic mice.

Tokio Matsuzaki¹, Oscar Alvarez-Garcia¹, Yukio Akasaki², Natalia Reixach¹, Joel Buxbaum¹, Martin K. Lotz¹

¹Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA, USA; ²Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

jbux@scripps.edu

INTRODUCTION: Deposition of amyloid is a common aging-associated phenomenon with wild type human TTR being the most common systemic precursor. Cardiac, carpal tunnel and gastrointestinal TTR amyloid deposits have been observed with increasing frequency with increasing age. Amyloid deposits have been reported in osteoarthritic joints as far back as the 1970’s. In the first study of joint amyloid using precursor specific antisera in 1985, Goffin and colleagues reported that TTR amyloid was present in approximately a third of the joints obtained from patients with osteoarthritis. Since then a number of studies of OA in knees and hips have revealed that TTR associated amyloid is present in approximately one third of the tissues examined. Recently ApoA1 amyloid has been found with a significant frequency and at least one laboratory has noted a significant number of samples in which the amyloid precursor could not be identified. In our published work we have reported that all of our elderly (>68 years of age) subjects with osteoarthritis had TTR amyloid deposits in the affected joints. We also found that the majority of elderly subjects without histologic evidence of OA had both amyloid and non amyloid TTR deposits in their joint cartilage. These findings raised several important questions. Does TTR joint deposition lead to osteoarthritis? Does the weight-bearing joint space provide a permissive environment for TTR amyloid formation? Is the osteoarthritic joint milieu amyloid enhancing? If any or all of these are true what is (are) the molecular mechanism(s) and what do they tell us about TTR amyloid deposition in general?

MATERIALS AND METHODS: OA was surgically induced by destabilizing the medial meniscus of 4 month old hTTR TG (n=26) and control mice (n=22). Mice were sacrificed 10 weeks after surgery. To investigate the effects of TTR on cartilage with joint damage, 6-month-old mice were sacrificed and tissues examined by immunohistochemistry, real-time PCR and Western blotting. In addition, quantitative analysis of cell number in cartilage was examined in the joints of 6 and 12-month-old normal and surgical model mice. TTR in cartilage and chondrocytes was analyzed by immunohistochemistry and Western blotting. OA-related tissue changes were evaluated using the Glasson’s semi-quantitative cartilage scoring system and Krenn’s synovitis score.

RESULTS: TTR protein was detected in the knee cartilage of over-expressing hTTR TG mice, but, as in human joints and cultured chondrocytes, there was no increase in chondrocyte TTR mRNA synthesis. ADAMTS4 and MMP13 mRNA's were significantly elevated in cartilage in 6 month-old hTTR TG mice compared with controls but there was no histologic evidence of OA. In the surgical model immunohistochemical and Western blotting analysis showed increased MMP13 expression in the hTTR TG mice 10 weeks after surgery compared with control mice. In addition, nuclear factor-kB (NF-kB) p65 and Phospho-NF-kB p65 were elevated in the hTTR TG mice. Both histological OA and synovitis scores were significantly higher in the hTTR TG mice. Additionally, cellularity was significantly lower in the 6-month-old hTTR TG mice and hTTR TG mice with post-surgical OA compared with control mice.

CONCLUSIONS: These findings are the first to show that TTR deposition accelerated the development of OA in the surgically-induced murine OA model. TTR amyloid formation represents a novel mechanism that contributes to aging as a risk factor for OA and may represent a new target for OA prevention and treatment.
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**Systebryl™ (PTI-110) is a novel small molecule that modulates aggregation of patient derived wild type TTR amyloid (ATTRwt) and stabilizes TTR in plasma**

L Esposito¹, L. Connors², N. Ferreira³, M.R. Almeida³, M. Saraiva³ and K. Duchin¹

¹ProtaMed, Inc., Kirkland, WA, USA. ²Amyloidosis Center, Boston University, USA. ³Institute for Molecular and Cellular Biology, University of Porto, Portugal.

luke.esposito@protamed.us

**INTRODUCTION:** Systebryl™ (PTI-110) is a proprietary small molecule that targets specific amyloid proteins, including immunoglobulin light chain amyloid and the inflammation-associated amyloid A. The potential of PTI-110 as a treatment for wild-type transthyretin (TTR) ATTRwt amyloidosis and familial amyloidotic polyneuropathy (FAP) is established by demonstrating potent anti-aggregation activity using *ex vivo* ATTRwt isolated from two patients and recombinant TTR (rTTR) with the FAP-associated Leu55Pro mutation (ATTRL55P). The effect of PTI-110 on levels of the TTR homotetramer *in vivo* were determined in adult TTR Val30Met transgenic mice.

**MATERIAL & METHODS:** Congo red (CR)-positive ATTRwt was isolated from the heart of two unrelated male patients (75 and 76 years of age). Neither subject had any evidence of plasma cell dyscrasia and the TTR gene from both was mutation free. Complementary assays, including the CR binding assay, Thioflavin T (ThioT) fluorometry, and Thioflavin S (ThioS) staining were used to assess ATTRwt at 1, 3 and 7 days of incubation with PTI-110. In addition, rTTR was used in the ThioT fluorometry assay and assessed by electron microscopy (EM) to show that PTI-110 reduces ATTRL55P aggregate levels. A single dose at levels 10, 25 and 50 mg/kg PTI-110 and vehicle was administered subcutaneously to groups of 6-8 mice per dose level. At 6 hr and 24 hr after injection, plasma levels of TTR tetramer were assessed using isoelectric focusing. TTR tetramer levels were expressed as a percentage of total TTR.

**RESULTS:** PTI-110 reduces ATTRwt fibrils in a dose-dependent manner as assessed by the ThioT fluorometry and CR binding assays. In 2 patients, the approximate IC50 value after 1 day of exposure to PTI-110 was 0.01:1 (PTI-110: ATTRwt, w/w), with >70% reduction at 0.03:1 PTI-110:ATTRwt. Similar results were noted with the CR binding assay. To assess whether PTI-110 reduces ATTRL55P aggregates formed *in vitro*, recombinant ATTRL55P was pre-aggregated and PTI-110 was added to the mixture. ThioT fluorometry demonstrates that PTI-110 reduces ATTRL55P aggregate levels after 1 day of incubation in a dose-dependent manner with an IC50 of about 1:1. The effect is sustained for 15 days, with results confirmed by EM. In addition to reducing ATTR, single doses of PTI-110 (25 and 50 mg/kg) stabilized the TTR tetramer by 28% and 44 % at 6 hr post dose, respectively, and by 24% and 28% at 24 hr post-dose, respectively, relative to vehicle-treated controls.

**DISCUSSION & CONCLUSIONS:** PTI-110 reduces ATTR that is formed from wild type TTR isolated from human heart, and rTTR with the FAP-associated L55P mutation. PTI-110 thus targets both wild type and mutant TTR, thereby offering a potential advantage over therapeutics that target only a specific TTR mutant form. Stabilization of TTR occurred with single doses of PTI-110 *in vivo*. 
Identification of a new variant of TTR involved in familial amyloid cardiomyopathy (FAC) in Brazil: from the patient to the protein

Priscila Ferreira¹, Cinthia Lima¹, Antonio Pereira-Neves², Humberto Pereira³, Fernando Palhano¹, Amanda Berensztejn³, Roberto Pedrosa¹, Márcia Cruz³, Debora Foguel¹.

¹-Instituto de Bioquímica Médica Leopoldo De Meis, Universidade Federal do Rio de Janeiro, Brazil. ²- Fiocruz Pernambuco, Centro de pesquisa Aggeu Magalhães, Departamento de Microbiologia, Brazil. ³- Instituto de Física de São Carlos, Universidade de São Carlos, Brazil. 4- Centro de Estudos de Paramiloidose Antônio Rodrigues de Mello, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Brazil.

Introduction: In Brazil, the most prevalent cases of TTR-related amyloidoses is the V30M variant due the Portuguese colonization. Our group has stablished a center for molecular diagnostic of FAP. Since then, we have sequenced almost one hundred patients form the University Hospital and their relatives. Recently, we identified a patient with a severe cardiomyopathy. This patient has a German ancestry and the sequence of his TTR gene revealed the presence of a new mutation, namely A19D. This patient presented heart failure and was classified by the NIH as IV. We have also identified a patient, 66-years old, from a family with African ancestry, which bears the typical V122I mutation. This patient presented carpal tunnel syndrome and two years later developed heart failure that progressed to NYHA III. The main goal of the present work is to characterize the Brazilian population with FAC by combining bioinformatics and biophysical studies.

Material and methods: We used HHP denaturation for evaluate the thermodynamic stability and aggregation assay for characterize the amyloidogenic profile of A19D. The viability assay was done in primary culture of cardiomyocytes and fibroblast.

Results: Our data show that A19D is a dimer and not a tetramer as the most TTR variants. The crystallographic structure of A19D is identical of wild type TTR but some differences in residues distances and orientation causing changes in non covalent contacts. Thermodynamic studies with A19D indicated that it has a lower stability than the wild-type protein and other mutants. This new mutant has a faster aggregation kinetics forming amyloid fibers in two hours as shown by images. Amyloid aggregates of A19D and V122I were incubated with primary culture of cardiomyocytes and fibroblasts from murine heart. The viability assay showed that the oligomers of A19D and V122I are toxic for cardiomyocytes and neuroblastoma cells and interestingly fibroblasts also suffer injury in the presence of these aggregates.

Discussion and Conclusions: The recent consolidation of TTR diagnosis in our University Hospital led to the identification of a rare, new variant of TTR in Brazil, namely, A19D, as well as the common V122I variant. A19D presented a marginal thermodynamic stability as inferred by bioinformatics and by biophysical studies with the purified protein. A19D showed to be dimer in solution. The viability assay shows that toxic mechanism displayed by this new mutant can be directly correlated with the aggressiveness observed in the disease developed by the patient.
Renal manifestations in hereditary transthyretin amyloidosis.

Asuncion Ferrer-Nadal1, Cristina Gallego-Lezaun,2, Manuel Raya-Cruz2, Mercedes Uson3, Antoni Figuerola3, Carles Montala4, Cristina Descals4, Tomas Ripoll5, Juana Nuñez5, Hernan Andreu6, Eugenia Cisneros-Barroso2, Juan Buades2.

1Nephrology Department, Hospital Son Llàtzer, Palma de Mallorca, Spain. 2Internal Medicine Department, Hospital Son Llàtzer. Palma de Mallorca. Spain 3Neurology Department. Hospital Son Llàtzer. Palma de Mallorca. Spain. 4Neurophysiology Unit, Hospital Son Llàtzer, Palma de Mallorca, Spain. 5Cardiology Department, Hospital Son Llàtzer, Palma de Mallorca, Spain. 6Gastroenterology Department, Hospital Son Llàtzer, Palma de Mallorca, Spain. mariaasuncion.ferrernadal@hsll.es

INTRODUCTION: Transthyretin-associated amyloidosis (ATTR) is an autosomal-dominant disease frequently associated with the substitution of methionine for valine at position 30. Peripheral neuropathy and autonomic dysfunction are often the first manifestations of this rare disease. Kidney disease has recently reported by the Portuguese group as a result of renal deposition. They identified in the largest series of patients published in recent years that one third of patients developed proteinuria and 10% progressed to End Stage Renal Disease (ESRD). Mallorca presents one of the most important focus of ATTR in Europe.

METHODS: This is a retrospective and prospective study of ATTR V30M carriers. Renal assessment included clinical and laboratory tests in blood and urine.

RESULTS: 132 ATTR V30M carriers were recruited at the Hospital Son Llátzer among 2002 and 2015. Mean age at the onset was 48.64 years (SD 16.2), median 46.5 (IQR 35.2-62.7). 52% male. 26 cases (19.7%) presented Chronic Kidney Disease (CKD) MDRD < 60 ml/min and 12 cases (9%) with severe CKD (<30 ml/min), 18 patients (7%) were found to excrete microalbuminuria, 11 patients (8.3%) proteinuria. 3 patients are on Renal Replacement Therapy. 4 patients with severe CKD developed proteinuria. 19 (14.4%) patients died during the study period. Other studies and actualization of data during 2016 will be presented at the poster.

CONCLUSIONS: We have observed in our patients 2 different phenotypes of ESRD, one proteinuric and another not proteinuric, suggesting that the patients develop ESRD by totally different pathophysiologic mechanisms.


Fig1. Clinical outcomes among proteinuric and non-proteinuric cases.
INTRODUCTION: Diflunisal is a well known Food and Drug Administration-registered commonly used nonsteroidal anti-inflammatory drug (NSAID) therapy in the United States of America since the 1970's. In Europe, the drug has been seldom authorised on a national basis with the next safety update report scheduled for 2025 (http://www.ema.europa.eu/ema/). Spain is one such country where commercial use has not been authorised, likely because of concerns on liver hypersensitivity and availability of other NSAIDs. Interestingly, recent advances have shown a potential beneficial effect in hereditary transthyretin amyloidosis (ATTR), as evidenced by encouraging data from the diflunisal trial consortium [1] where quality of life, neuropathy impairment scores and nutritional status showed significant, though modest, better results in patients randomised to receive diflunisal instead of placebo.

MATERIAL & METHODS: We aimed at describing the first off-label (compassive) use of diflunisal in a small cohort of 10 patients affected by variable degrees of TTR-familial amyloidotic polyneuropathy (FAP) in our centre. Inclusion criteria consisted of any symptomatic hereditary ATTR patient with progression of FAP either unfit or unwilling to receive either liver transplantation (LT)/Vyndaqel® as per on-label indication or to enter an ongoing clinical trial (e.g. RNA silencing). Basal demographic and clinical characteristics were collected. Follow-up was performed every 2 months with particular attention to disease progression “red flag” signs (polyneuropathy, dysautonomy, ECG) and incidence of adverse events.

RESULTS: A total of 10 patients were followed-up for a median 15.5 months [interquartile range (IQR 8.75)]. No deaths were reported. Seven patients (70%) reached at least 12-month follow-up (median 18 months; IQR 4.5 months). Median Karnofsky scores (67) decreased non-significantly by 3 points. Neuropathic pain improved in 5 (50%) patients. Overall, modal score for polyneuropathy disability score (PND), Coutinho staging and clinical FAP-Kumamoto score [2,3] did not show a significant change (scores of II, 2 and 37.2 respectively). Mean modified body-mass index at baseline (952 kg·g/L·m^2) [4] improved non significantly by 7.1 kg·g/L·m^2. Only one patient progressed from PND-II to PND-IIIA score. Clinical FAP-Kumamoto score increased non significantly by 1.6 points during mean follow-up period. All patients had at least one adverse event (AE). Mean time to first AE was 2.2 months (range 0.5-4 months). Acute renal failure (defined as a ≥0.3mg/dL rise in serum creatinine) was the most frequent AE, occurring transiently in 5 patients (one acute pyelonephritis, four recovering with diflunisal dose adjustment). Overall eGFR (79.5ml/min/1.73m^2) and urine protein/creatinine ratio did not significantly change. Three patients (30%) were discontinued after a median of 17 months (range 8-18) because of either persistent carpal tunnel syndrome, or disease progression (congestive heart failure, haemorrhagic stroke). All other AEs were mild and related to disease activity.

DISCUSSION & CONCLUSIONS: Diflunisal off-label (compassive) use in selected cases of evolving TTR-FAP patients who are not candidates for other therapies seems to be a relatively safe and effective option. Particular attention however must be paid to renal function as well as ominous signs of progressive ATTR in this fragile population as dose adaptation or drug discontinuation and shift to other putative therapies may be warranted.

Mass spectrometry analysis of transthyretin post-translational modifications in hereditary transthyretin amyloidosis: a case-control Spanish experience

Marta Vilà-Rico¹, Sebastián Azorín², José E Barcena Llona³, Ricardo Rojas-García⁴, Fernando Martínez Valle⁵, Antoni Planas¹, Francesc Canals⁶, Josep M Campistol².

¹Institut Químic Sarrià, Universitat Ramón Llull – Laboratory of biochemistry, Barcelona, Spain. ²Amyloidoses and Monoclonal Gammopathies’ Unit (UDAM). - Nephrology and transplant unit (SNiTR), Hospital Clinic de Barcelona, Spain. ³Multiple sclerosis and demyelinating diseases unit - Neurology Service, Department of Neurosciences, Cruces University Hospital, Biscay, Spain. ⁴Neuromuscular disease unit – Neurology Service, Hospital de la Santa Creu I Sant Pau, Barcelona, Spain. ⁵Internal medicine department I – Vall d’Hebrón University Hospital, Barcelona, Spain. ⁶Proteomics laboratory – Vall d’Hebron Institute of Oncology, Barcelona, Spain.

azorin@clinic.ub.es

INTRODUCTION: Transthyretin (TTR) is an amyloidogenic tetrameric protein, present in human plasma, associated with several familial amyloidoses. Variability of TTR is not only due to point mutations in the encoding gene but also to post-translational modifications (PTMs) at Cys10, being the most common PTMs the S-sulfonation, S-glycinylcysteinylation, S-cysteinylation and S-glutathionylation. It is thought that PTMs at Cys10 may play an important biological role in the onset and pathological process of the amyloidosis. Recently we reported the development of a methodology for quantification of PTMs in serum samples, as well as for the determination of serum TTR levels, from healthy (wild type-TTR) and transthyretin amyloidosis (ATTR) Val30Met (V30M) individuals which involves an enrichment step by immunoprecipitation followed by mass spectrometry analysis of (i) the intact TTR protein and (ii) targeted LC-MS analysis of peptides carrying the PTMs of interest [1]. Analysis of serum samples by the combination of the two methods affords complementary information on the relative and absolute amounts of the selected TTR PTM forms.

MATERIAL & METHODS: We aimed at describing the applicability of our mass spectrometry methodology among healthy controls and V30M-TTR patients (cases) at different disease stages followed at our institution and other three Spanish health facilities. Inclusion criteriae for cases consisted of a positive genetic testing for V30M-TTR status and a signed ethics’ committee approved informed consent. Mass spectrometric analysis was performed as detailed in our previous work.

RESULTS: A total of 39 healthy controls and 29 patients (sixteen asymptomatic; thirteen symptomatic with five (39%), six (46%) and two (15%) classified as Coutinho stages 1, 2 and 3 respectively) were included after signing the informed consent. Demographic and clinical characteristics were gathered and correlated to the proteomic analysis profile according to our validated methodology. Significant differences were found for total TTR as well as for specific TTR Cys-10 PTMs between the different V30M ATTR stages as well as between controls and symptomatic patients. Particularly, TTR levels were significantly higher among TTR cases compared to healthy controls; no differences were found between the different stages in the V30M population. V30M circulating TTR ranged 60% of all circulating TTR and this was so independently of symptomatic status or disease stage. Concerning Cys-10 PTMs, S-glycinylcysteinylation and S-glutathionylation had a significant tendency to vary between the control population and also between the earliest stages of ATTR progression.

DISCUSSION & CONCLUSIONS: Quantification of wt:V30M TTR ratio and quantification of Cys-10 PTM isoforms is a feasible, reproducible and robust method by intact TTR and targeted LC-MS in the TTR V30M population. Significant differences for wt:V30M TTR ratio as well as for some specific PTMs have been found in a small cohort of V30M-TTR patients at different ATTR stages. These results need to be confirmed in a bigger cohort of patients with ATTR.

Investigating partially unfolded poorly populated conformations of human transthyretin

SA Ghadami, F Tramontana, S Conti, C Cecchi, F Chiti, F Bemporad

1 Department of Clinical and Experimental Biomedical Sciences, University of Florence, Viale G. B. Morgagni 50 – 50134 Firenze (Italy)
francesco.bemporad@unifi.it

INTRODUCTION: Transthyretin (TTR) is a homotetrameric protein whose misfolding and deposition is linked to localized and systemic amyloidoses. Recently, it has been shown that TTR is able to exert a protective role against aggregation of the Aβ peptide, a process which has been linked to Alzheimer’s disease. In vitro, these processes correlate with the ability of TTR to convert into its monomeric state. In order to achieve a complete description of the conformations populated, possibly transiently, by this protein and of their role with respect to the processes mentioned above, we undertook a characterization of the folding/unfolding pathway of a monomeric variant (F87M/L110M) of TTR (Jiang et al., 2001).

MATERIAL & METHODS: The F87M/L110M variant of TTR was purified as previously reported (Jiang et al., 2001) or by exploiting a new construct which allows TTR to be purified by means of affinity chromatography. A battery of biophysical methods was employed to investigate folding/unfolding equilibria and kinetics. In brief, urea induced titration curves were combined with stopped-flow kinetics and T-jump relaxation experiments. Cysteine labelling with a coumarinic probe is currently being exploited to investigate refolding by means of intramolecular FRET between tryptophan and coumarin.

RESULTS: As depicted below, we characterized the folding equilibrium of monomeric TTR and identified a set of partially folded conformations in equilibrium with the folded monomeric (F, see below, Figure 1). Before the unfolded state (U) can convert into F, a sub-population of the protein molecules convert into an off-pathway partially folded conformation (PF), possessing secondary structure comparable to that of F, as assessed by means of circular dichroism and FRET. One further subpopulation folds slower, due to the occurrence of proline isomerism. Furthermore, a non-cooperative transition leads to the formation of a molten globular state (MG) at denaturant concentrations lower than those required to induce full unfolding.

DISCUSSION & CONCLUSIONS: By comparing data collected at physiological pH with experiments carried out at acidic pH, i.e. under conditions that promote aggregation of TTR, we are currently in the process of investigating possible biological roles played by the PF and MG states. The identification and the characterization of the conformational states here described represents a first step towards a better understanding of the properties of TTR in solution.

REFERENCES:


Figure 1: Conformational equilibria of monomeric transthyretin; 4F = tetrameric TTR; F = monomeric folded TTR; MGn = molten globular TTR; U(t) = unfolded TTR with proline in a trans configuration; U(c) = unfolded TTR with proline in a cis configuration; PF(t) = partially folded TTR with proline in a trans configuration; PF(c) = partially folded TTR with proline in a cis configuration; reprinted from Conti et al.
**PA80**

**Phase 2 open-label extension study of patisiran, an investigational RNAi therapeutic for the treatment of hereditary ATTR amyloidosis with polyneuropathy**

OB Suhr¹, T Coelho², I Conceicao³, M Waddington Cruz⁴, H Schmidt⁵, J Buades⁶, J Campistol⁷, J Pouget⁶, J Berk⁸, M Polydefkis⁹, N Ziyadeh¹¹, AM Partisano¹¹, J Chen¹¹, J Gollob¹¹, D Adams¹²

¹Umeå University, Umeå, Sweden. ²Hospital de Santo António, Centro Hospitalar do Porto, Porto, Portugal. ³Centro Hospitalar Lisboa Norte-Hospital de Santa Maria, Lisbon Portugal. ⁴Hospital Universitário Clementino Fraga Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. ⁵Universitätsklinikum Münster, Münster, Germany. ⁶Hospital Son Llatzer, Palma de Mallorca, Spain. ⁷Hospital Clinic, University of Barcelona, Barcelona 8036, Spain. ⁸Hôpital de La Timone, Marseille, France. ⁹Boston University, Boston, USA. ¹⁰Johns Hopkins University, Baltimore, USA. ¹¹Alnylam Pharmaceuticals, Cambridge, USA. ¹²National Reference Center for FAP (NNERF)/ APHP/ INSERM U 1195/ CHU Bicêtre, Le Kremlin-Bicêtre, France.

**Introduction:** Hereditary ATTR amyloidosis with polyneuropathy (hATTR-PN), also known as familial amyloidotic polyneuropathy (FAP), is an inherited, progressive disease leading to death within 5 to 15 years. It is due to a mutation in the transthyretin (TTR) gene, which causes misfolded TTR proteins to accumulate as amyloid fibrils predominantly in peripheral nerves and other organs. hATTR-PN can cause sensory, motor, and autonomic dysfunction, resulting in significant disability and death. Patisiran is an investigational, systemically-administered lipid nanoparticle (LNP) formulated RNA interference (RNAi) therapeutic targeting wild-type and mutant TTR. This formulation is delivered predominantly to the liver, thereby inhibiting synthesis of TTR at the primary site of production. A multi-center, multi-dose Phase 2 trial of patisiran in patients with hATTR-PN showed >80% sustained mean knockdown of serum TTR when administered at a dose of 0.3 mg/kg every 3 weeks.

**Materials and Methods:** A Phase 2 open-label extension (OLE) study of patisiran in patients with hATTR-PN, who participated in the aforementioned Phase 2 trial, was initiated in October 2013 (NCT01961921). The primary objective of the study is to evaluate the safety and tolerability of patisiran. Secondary objectives include assessment of patisiran’s effect on serum TTR levels, as well as evaluation every 6 months of its impact on clinical measures, including the mNIS+7 composite neuropathy impairment score and quality of life (QOL).

**Results:** Twenty-seven patients were enrolled; median age 64 years (range: 29-77 years). Interim data presented indicate chronic dosing with patisiran has been generally well tolerated out to 25 months (data-cutoff: Feb 2016). Five patients experienced serious adverse events unrelated to study drug, including one discontinuation for gastroesophageal cancer (death). A second unrelated death due to myocardial infarction occurred post data-cutoff. The most common related adverse events were flushing (22.2%) and infusion-related reactions (18.5%), which were mild in severity and did not result in any discontinuations. Sustained mean serum TTR lowering of approximately 80% was achieved for over 24 months, with mean maximal knockdown of 92%. Neuropathy impairment scores were stable through 18 months with a mean change in mNIS+7 and NIS of -0.8 and 2.6 points, respectively; this compares favorably to a 17-26 point increase in mNIS+7 or NIS estimated at 18 months from prior hATTR-PN studies in a patient population with similar baseline neurologic impairment. Additionally, a significant improvement of distal thigh sweat gland nerve fiber density was observed, with a median increase of 4.5 m/mm³ (~77% increase from baseline) at 18 months (P < 0.001).

**Discussion and Conclusion:** Interim 18-month data from this Phase 2 OLE study demonstrate that long-term administration of patisiran was generally well tolerated, resulted in robust and sustained serum TTR lowering, and is consistent with the therapeutic hypothesis that TTR knockdown has the potential to halt neuropathy progression. Interim 24-month results from this ongoing study will be presented.

Carpal tunnel biopsy: A diagnostic tool in Cardiac Amyloidosis

T Youngstein¹, E Gillott², J Gilbertson¹, D Hutt¹, T Lane¹, D Rowczenio¹, T Rezk¹, C Quarta¹, JD Gillmore¹, C Whelan¹, HJ Lachmann¹, N Goddard², PN Hawkins¹.

¹National Amyloidosis Centre, Division of Medicine, Royal Free Campus, University College London, UK
²Department of Orthopaedics, Royal Free London NHS Foundation Trust, London, UK

t.youngstein@ucl.ac.uk

Introduction:
Other than cardiac amyloidosis, carpal tunnel syndrome is the predominant clinical manifestation of non-hereditary wild type ATTR amyloidosis. In our cohort of patients with proven cardiac ATTR amyloidosis, 98% had evidence of median nerve entrapment on neurophysiological testing and 48% had a history of carpal tunnel decompression as much as 12 years prior to clinical presentation with heart failure symptoms.

Carpal tunnel syndrome is common in the general population, but tissue is seldom obtained at surgical decompression for histological examination. We wished to explore the role of carpal tunnel histology in making a diagnosis of systemic amyloidosis at an early stage of its course to inform the design of a long term follow-up study.

Materials & Methods:
Carpal tunnel biopsies were taken at decompression surgery from individuals with proven moderate to severe median nerve entrapment. Biopsies were stained with Congo red and viewed under cross polarised light. Immunohistochemistry was used to type any amyloid deposits that were present.

Results:
We analysed 21 carpal tunnel biopsies, 76% female, mean age 65 yrs (35-87 years). 3 biopsies contained definite amyloid deposits (14%) and one further case was equivocal. Of the 3 definite cases, 2 were typed as ATTR amyloid using immunohistochemistry (9.8% of total cohort, male: female 50%), and one demonstrated no specific staining. Proteomic analysis is underway.

Neither case with proven ATTR amyloid had a history of heart failure symptoms at the time of carpal tunnel decompression. One of these patients, a 74 year old male, attended the NAC for further diagnostic work up. He had wild-type TTR gene sequence and a normal ECG and echocardiogram but Tc-DPD scintigraphy demonstrated uptake of tracer within the intraventricular septum and left ventricular wall on CT-SPECT indicative of early cardiac amyloidosis. The patient declined cardiac MRI.

Discussion & Conclusions:
Carpal tunnel biopsy provides a simple and accessible opportunity to identify and type amyloid deposits and may identify those at risk of developing cardiac ATTR amyloidosis at an early and potentially more treatable stage. This pilot study has informed the design of a large multicentre prospective study to identify the UK prevalence of ATTR amyloid in patients with carpal tunnel syndrome. Long term detailed follow up of this large cohort of 250 patients with 6 MW, Tc-DPD scanning and cardiac MRI will elucidate the natural history of ATTR amyloidosis and potentially permit early intervention with the disease-modifying therapies currently in development.
Patisiran, an investigational RNAi therapeutic for the treatment of hereditary ATTR amyloidosis with polyneuropathy: Baseline demographics from the phase 3 APOLLO study

D Adams1, A Gonzalez-Duarte2, W O’Riordan3, CC Yang4, T Yamashita4, A Kristen6, I Tournev7, H Schmidt8, T Coelho9, J Berk10, KP Lin11, J Chen12, J Gollob12, and OB Suhr13 on behalf of the APOLLO investigators

Introduction: Hereditary ATTR amyloidosis with polyneuropathy (hATTR-PN), also known as familial amyloidotic polyneuropathy (FAP), is an inherited, progressive disease leading to death within 5 to 15 years. It is due to a mutation in the transthyretin (TTR) gene, which causes misfolded TTR proteins to accumulate as amyloid fibrils predominantly in peripheral nerves and other organs. Hereditary ATTR amyloidosis with polyneuropathy can cause sensory, motor, and autonomic dysfunction, resulting in significant disability and death. Patisiran is an investigational, RNA interference (RNAi) therapeutic targeting mutant and wild-type TTR. Previously reported data from the patisiran Phase 2 and Phase 2 open-label extension (OLE) studies showed a >80% sustained mean knockdown of serum TTR and that patisiran was generally well tolerated in patients with hATTR-PN.1,2 Additionally the OLE study demonstrated evidence for potential disease stabilization at 18 months2. The patisiran Phase 3 APOLLO trial completed enrollment; the study is ongoing. The objective of this abstract is to present the baseline demographics of patients enrolled in APOLLO.

Materials and Methods: APOLLO is a Phase 3 multi-center, international, randomized, double-blind, placebo-controlled study (NCT01960348) designed to evaluate the efficacy and safety of patisiran in patients with hATTR-PN. Symptomatic patients with a neurological impairment score (NIS) of 5-130 were eligible for enrollment. Select exclusion criteria included: prior liver transplantation, current tetramer stabilizer use, PND score >3b, and NYHA class >2. Patients were randomized 2:1 to receive an intravenous (IV) infusion of patisiran 0.3mg/kg or placebo once every 3 weeks. The primary endpoint of the study is the change from baseline at 18 months in the mNIS+7 composite neurologic impairment score, which consists of the modified NIS tool (weakness and reflexes), NCS 5 attributes (NCS Σ5), quantitative sensory testing (QST) by body surface area including touch pressure and heat pain, and postural blood pressure. Secondary endpoints include assessments of quality of life and changes in mBMI, motor and autonomic function.

Results: A total of 225 patients with hATTR-PN were enrolled at 44 sites in 19 countries from December 2013 to January 2016 and representative of the global patient population (51% of patients from Europe; 21% from North America; 20% from Asia Pacific and 8% from Latin America). Median age was 62 years (range: 24-82), 74% were males, 42% had the Val30Met TTR mutation, 58% had non-V30M mutations (including 73 different TTR genotypes), and 53% were previously treated with a TTR tetramer stabilizer (tafamidis or diflunisal). Measures of baseline disease severity showed: 46% FAP Stage 1, 53% FAP Stage 2; and mean mBMI of 978.7 kg/cm²g/L (range: 522.1-1530.0). Mean baseline NIS and mNIS+7 were 59.3 (range: 6.0-141.6) and 78.8 (range: 8.0-165.0), respectively. Echocardiographic evidence of cardiac involvement (LV wall thickness ≥ 13 mm) was present in 54% of patients. These patients had a mean NT-proBNP and troponin levels of 1461 ng/L (range: 40-7895) and 0.1 ng/mL (range: 0.1-1.0), respectively.

Discussion and Conclusions: An overview of the baseline demographics demonstrate that the Phase 3 APOLLO study enrolled patients with hATTR-PN with a wide range of TTR genotypes and neuropathy severity, including a substantial proportion of patients with cardiac involvement. This is the largest, controlled study of patients with hATTR-PN to date and is representative of the global patient population.

Relationship between TTR knockdown and change in mNIS+7: Preliminary correlation findings from the phase 2 open-label extension study of patisiran, an investigational RNAi therapeutic for hereditary ATTR amyloidosis with polyneuropathy

T Coelho¹, OB Suhr ², I Conceicao³, M Waddington Cruz⁴, H Schmidt⁵, J Buades⁶, J Campistol⁷, J Pouget⁸, J Berk⁹, N Ziyadeh¹⁰, AM Partisano¹⁰, J Chen¹⁰, J Gollob¹⁰, D Adams¹¹

¹ Hospital de Santo António, Centro Hospitalar do Porto, Porto, Portugal. ² Umeå University, Umeå, Sweden. ³ Centro Hospitalar Lisboa Norte-Hospital de Santa Maria, Lisbon Portugal. ⁴ Hospital Universitário Clementino Fraga Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. ⁵ Universitätsklinikum Münster, Münster, Germany. ⁶ Hospital Son Llatzer, Palma de Mallorca, Spain. ⁷ Hospital Clinic, University of Barcelona, Barcelona 8036, Spain. ⁸ Hôpital de La Timone, Marseille, France. ⁹ Boston University, Boston, USA. ¹⁰ Alnylam Pharmaceuticals, Cambridge, USA. ¹¹ National Reference Center for FAP (NNERF), APHP, INSERM U 1195, CHU Bicêtre, Le Kremlin-Bicêtre, France.

Introduction: The deposition of liver-derived circulating transthyretin in peripheral nerves is central to the pathophysiology of hereditary ATTR amyloidosis with polyneuropathy (hATTR-PN, also known as familial amyloidotic polyneuropathy [FAP]). It is hypothesized that the reduction in circulating TTR levels will reduce TTR amyloid deposition and could potentially lead to stabilization and/or improvement of disease in patients with hATTR-PN. Patisiran, an investigational RNAi therapeutic targeting hepatic production of wild-type and mutant TTR, is being studied in patients with hATTR-PN. Previously reported data from the patisiran Phase 2 and Phase 2 open label extension (OLE) study showed a >80% sustained mean knockdown (KD) of serum TTR. We undertook an evaluation of the relationship between the degree of TTR KD by patisiran and change in neurologic impairment over time in patients treated on the Phase 2 OLE study.

Materials and Methods: A Phase 2 OLE study of patisiran in patients with hATTR-PN was initiated in October 2013 (NCT01961921). The primary objective of the study is to evaluate the safety and tolerability of patisiran. Secondary objectives include assessment of patisiran’s effect on serum TTR levels, as well as evaluation every 6 months of its impact on clinical measures, including the mNIS+7 composite neurologic impairment score. Correlation between TTR KD and changes in mNIS+7 was estimated using Pearson correlation coefficient. TTR KD 17 days post-first dose of patisiran (Day 17 %TTR KD) was chosen for analysis of correlation between TTR KD and change in mNIS+7 at 6, 12 and 18 months. Day 17 %TTR KD correlates with TTR area under the curve (AUC) and mean %TTR KD (r > 0.85, p < 0.0001), and its use reduces the impact of missed doses or missed TTR assessments on the measurement of patisiran’s effect on serum TTR burden over 18 months of dosing.

Results: The ongoing Phase 2 OLE enrolled 27 patients with a median age of 64 years (range: 29-77 years). Interim data² indicated chronic dosing with patisiran has been generally well tolerated out to 25 months. Within the OLE study, sustained mean serum TTR lowering of approximately 80% was achieved for over 24 months, with mean maximal KD of 92%. Eighteen month data showed stabilization of neuropathy impairment scores with a mean change in mNIS+7 and NIS of -0.8 and 2.6 points, respectively. Correlation analyses demonstrated a significant relation between Day 17 %TTR KD and change in mNIS+7 from baseline at month 6 (r = -0.49, p=0.0099) and month 12 (r = -0.54; p=0.0034) and a trend at month 18 (r = -0.37; p=0.055).

Discussion and Conclusion: Interim data from the patisiran Phase 2 OLE study in hATTR-PN demonstrate that TTR KD correlates with change in mNIS+7. This analysis provides the first reported correlative evidence that the degree of TTR KD is associated with improvements in mNIS+7 and supports the therapeutic hypothesis that reduction of mutant and wild-type TTR may be associated with potential clinical benefit in patients with hATTR-PN.

Phase 2 open-label extension study of revusiran, an investigational RNAi therapeutic for the treatment of patients with transthyretin amyloidosis with cardiomyopathy: Updated interim results

JD Gillmore1, RH Falk2, MS Maurer3, M Hanna4, N Fine5, C Powell6, V Karsten6, J Vest6, J Gollob6, PN Hawkins1

1National Amyloidosis Centre, UCL, London UK; 2Amyloidosis Program, Brigham and Women’s Hospital, Boston, USA; 3Clinical Cardiovascular Research Lab, New York Presbyterian/Columbia, New York, USA; 4Heart and Vascular Institute, Cleveland Clinic, Cleveland, USA; 5Alberta Health Services, Calgary, CA; 6Alnylam Pharmaceuticals, Cambridge, USA

Background: ATTR amyloidosis is a progressive, life-threatening disease that often includes cardiomyopathy due to myocardial deposition and accumulation of liver-derived TTR amyloid fibrils and can result in heart failure and death within 2 to 5 years. Hereditary ATTR amyloidosis with cardiomyopathy (hATTR-CM), also termed familial amyloidotic cardiomyopathy (FAC), is an inherited form of the disease stemming from mutations in the TTR gene. Wild-type (wt) ATTR amyloidosis (wtATTR), also known as senile systemic amyloidosis (SSA), is the nonhereditary form of the disease caused by deposition of amyloid fibrils consisting of wt TTR leading to cardiomyopathy. Revusiran is a liver-directed subcutaneously administered investigational RNA interference (RNAi) therapeutic comprised of a GalNAc-siRNA conjugate targeting both mutant and wild-type TTR mRNA. A Phase 2 study in 26 patients with hATTR-CM and wtATTR demonstrated that weekly administration of revusiran was generally well tolerated and resulted in a greater than 85% sustained knockdown of serum TTR1.

Methods: A Phase 2 open-label extension (OLE) study of revusiran in patients with hATTR-CM and wtATTR was initiated in November 2014 (NCT02292186). The primary objective of the study is to evaluate the long-term safety of 500 mg revusiran administered as 5 daily doses for the first week followed by weekly dosing for 2 years. Secondary and tertiary objectives include serial assessments of pharmacodynamics (PD), clinical outcomes including 6-minute walk distance (6-MWD) and patient-reported quality of life (QoL).

Results: Among the 26 patients in the revusiran Phase 2 study, 25 patients enrolled in the OLE study; 11 patients with wtATTR and 14 patients with hATTR-CM [T60A: n=7; V122I: n=5; S77Y: n=1; I84S: n=1]; mean age: 70 years (range: 53-79); mean 6-MWD: 401 meters (range: 73–617); mean NT-proBNP: 3555 ng/L (range: 349–21310); mean troponin I: 0.14 ng/mL (range: 0.1–0.4). The majority of patients had mild or moderate heart failure with NYHA class II (68%) and III (24%) and all patients presented with intraventricular septal thickness (IVS) of > 15 mm (mean IVS 20 mm; range: 15-29). Interim 6-month data2 suggested weekly dosing with revusiran was generally well tolerated. Eight patients experienced serious adverse events unrelated to study drug, including 1 unrelated death due to infiltrative cardiomyopathy. Injection site reactions (ISRs) were the most common related adverse events. The majority of ISRs were mild in severity; ISR or diffuse rashes led to study discontinuation in 3 patients. Revusiran administration resulted in durable TTR knockdown with the mean maximum knock down > 85% following 6 months of treatment and was comparable between patients with hATTR-CM and wtATTR. Additionally, for patients with an evaluable 6-MWD measurement at 6 months (N=15), the majority exhibited stable performance compared to baseline. On average, evaluable patients with hATTR-CM exhibited a mean (± SEM) decline of 20 ± 14 meters and those with wtATTR exhibited a mean (± SEM) decline of 24 ± 20 meters over 6 months. No clinically meaningful changes were observed in additional cardiac parameters assessed, including NT-proBNP, troponin I and IVS thickness.

Conclusions: Six-month data from this Phase 2 OLE study demonstrated that long-term administration of revusiran was generally well tolerated and resulted in durable TTR lowering. Interim 12-month results highlighting the safety, PD and clinical outcomes of revusiran will be presented.

Transthyretin (ATTR) Amyloidosis nephropathy: lessons from a TTR stabilizer molecule

A Rocha1,2, Silva AM1,3, Cardoso M1,4, Beirão I1,5, Alves C1, Teles P6, Coelho T1,4, L Lobato1,2,5

1Unidade Corino de Andrade, Porto, Portugal. 2Unit for Multidisciplinary Research in Biomedicine-UMIB, Instituto de Ciências Biomédicas Abel Salazar-ICBAS, Porto University, Porto, Portugal 3Department of Neurology, Centro Hospitalar do Porto, Porto, Portugal 4Department of Neuropsychology, Centro Hospitalar do Porto, Porto, Portugal. 5Department of Nephrology, Centro Hospitalar do Porto, Porto, Portugal. 6School of Economics and LIAAD-INESC Porto LA, Porto University, Porto, Portugal

acrisbraga@gmail.com

INTRODUCTION: Tafamidis delayed neuropathic progression in patients with transthyretin amyloidosis (1), but long term effectiveness in multisystem commitment is unclear. In renal involvement, albuminuria represents the first stage of clinical nephropathy with progression to nephrotic proteinuria and end-stage renal disease as natural course of the disease (2). This report describes an open-label, nonrandomized, prospective evaluation of the effects of tafamidis on patients who had kidney transthyretin amyloidosis in terms of kidney function and reduction in proteinuria.

MATERIAL & METHODS: Twelve patients (11 female, 1 male), with neuropathy stage I and estimated glomerular filtration rate (eGFR) ≥60 mL/min, were treated with tafamidis, receiving 20 mg QD. Kidney function was evaluated by measuring creatinine and cystatin C. Proteinuria and urine creatinine were measured in a spot urine collection. Determinations and patient clinical evaluation were made at baseline and at all subsequent visits every 6 months. We also divided the patients into two groups on the basis of final proteinuria: 5 patients with proteinuria < 30 mg and 4 patients with proteinuria > 30 mg, and compare them. The unpaired Student’s T-test and the nonparametric Wilcoxon-Mann-Whitney test were used to determine the significance of differences.

RESULTS: Nine patients completed 36 months of therapy, 2 patients completed 30 months and 1 patient completed 18 months. The mean age was 51 years at the time tafamidis was begun and the mean duration of neuropathy was 5 years. Two patients had kidney biopsies, all had transthyretin amyloidosis. During the study period kidney function remained stable with sustained reductions in proteinuria (table 1). GFR calculated using CKD-EPI, MDRD and Cockcroft-Gault formulas was, respectively, at enrolment and at the end of follow-up of 98.8 and 98.4, 95.8 and 95.3, 83.1 and 80.5 ml/min. The patients with proteinuria > 30 mg had across all assessments higher significant values of creatinine and cystatin C.

DISCUSSION & CONCLUSIONS: Over the course of 36 months of tafamidis, there was no change in kidney function with sustained reduction in proteinuria. This suggests that tafamidis may slow or halt kidney disease demonstrating the feasibility of this therapy in patients with kidney transthyretin amyloidosis. It should be used before extensive kidney damage occurs as anticipated by the higher reduction of proteinuria in patients with lower levels of creatinine and cystatin C.


Table 1. Proteinuria and Urine protein/creatinine ratio.

<table>
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<tr>
<th></th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>t</th>
<th>p-value</th>
<th>W</th>
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<td>Proteinuria (mg)</td>
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<td></td>
<td></td>
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<tr>
<td>Month 36</td>
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<td>94.9</td>
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<td>Urine protein/creatinine</td>
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<td>0.002</td>
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<tr>
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<tr>
<td>Month 36</td>
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Familial amyloid polyneuropathy and hearing loss: the French cohort experience.

S Levivient¹, M Theaudin², J Ratovo¹, C Cauquil², C Lepajolec¹, D Adams², J Nevoux¹

¹ Department of Otolaryngology, Paris Saclay University, Le Kremlin-Bicetre, France. ² Department of Neurology, Paris Saclay University, Le Kremlin-Bicetre, France.
sarah.levivien@hotmail.fr

INTRODUCTION: Transthyretin familial amyloid polyneuropathy (TTR-FAP) is a rare autosomal dominant disease. In non endemic areas, there are many different TTR mutations, the Val30Met being the most common in France. Even though it is a systemic disease, the inner ear insult is very rarely reported in literature, with only very few case reports. In order to confirm inner ear involvement and to characterize the cochlear pathology in TTR-FAP, we decided to systematically screen the TTR FAP patients seen in the reference centre for inner ear involvement.

MATERIAL & METHODS: From June 2015 to April 2016, patients who were having their regular follow-up in the reference centre for their FAP and who signed a consent for the inner ear exploration were included in the cochlear exploration protocol. All patients had a complete ENT examination and a complete audiologic check-up including audiogram, auditory brainstem response (ABR) and otoacoustic emission (OAE). Results of those tests were compared to French reference data for hearing loss according to age. In order to take into account the normal age-related hearing loss, we divided patients in 3 categories to correlate their results with the standard hearing threshold of the presbycusis: under 60 years old (group 1) which is when presbycusis usually begins, between 60 and 70 years old (group 2) and over 70 years old (group 3). The study was approved by the local ethic board.

RESULTS: Nineteen patients were included. Mean age at examination was 60 years [24-81; SD]. All patients had the Val30Met TTR mutation except one with a Val22Ile mutation. There were 6 in group 1, 9 in group 2 and 4 in group 3. In group 1, including 6 patients, all patients were asymptomatic of systemic neurological signs except one (16,7%). The mean pure tone average (PTA) threshold was 10 dB, with a PTA threshold of 11 dB in the reference population of that age range. The ABR threshold was 35 dB [20-50 dB] with no evidence of neuropathy for any patient but the threshold was not normal reflecting a possible early sign of auditory involvement. The sensorial cells were slightly involved with OAE present in 80% of the patients. In group 2, including 9 patients, seven patients had peripheral neuropathy (77,8%). The PTA threshold was 33.5 dB, which is higher than in the reference population [24-31]. The mean threshold at 4 kHz was between 45 and 75 dB for 80% of the patients, much more than the 45 dB for the reference population. Similarly, the mean threshold in ABR was 56 dB [27-95 dB] with asynchrony of the waves suggesting a neuropathy. Of note, as OAE were absent in 72% of patients, the outer hair cells were also involved. In group 3, including 4 patients, all patients had peripheral neuropathy. The hearing loss was more significant with a mean PTA threshold of 64 dB, higher than in the reference population [31-36 dB]. The ABR threshold was increased in 75% of patients and OEA were absent in 100% of patients.

DISCUSSION & CONCLUSIONS: This study confirms that patients with TTR-FAP develop more severe hearing loss than the general population. This involvement of the hearing pathway could be due to amyloid deposits either in hair cells or in the auditory nerve. Further investigations are needed to better understand the mechanisms.
Ile68Leu transthyretin-related amyloidosis: a cardiogenic mutation endemic in central-northern Italy

M M Cinelli¹, F Cappelli², A Tinuper¹, S Ratti¹, C Gagliardi¹, M Lorenzini¹, S Foffi¹, A Milandri¹, C Rapezzi¹, F Perfetto²

¹ Cardiology, Department of Experimental, Diagnostic and Specialty Medicine – DIMES, University of Bologna, Italy ²Department of Internal Medicine, University of Florence, Italy.

INTRODUCTION: A limited number of transthyretin (TTR) mutations result in an exclusively or mainly cardiologic phenotype. While Ile122, Thr60 and Leu111 have already been analytically described, Ile68 that has been reported in the THAOS registry from Italian centres, has been the object of limited attention in literature. In this study we report a detailed analysis, including outcome data, from a large cohort of patients from two Italian referral centres. Patients with wild-type transthyretin (wt-TTR) from the same centres were used as a comparison group.

MATERIAL & METHODS: Fifty-six affected patients and 35 asymptomatic carriers (from 50 unrelated families) were evaluated at two centres (Bologna and Florence) between 1991 and May 2015. During the same period 82 patients with wt-TTR were followed.

RESULTS: Patients from the two centres were similar for all the relevant variables. Median age at diagnosis of affected patients was 71 [64-77], with 80% male prevalence. Disease penetrance was 50% at 70 years of age. The vast majority of patients had a prevalently cardiologic phenotype at presentation (exclusively cardiac in 73%), with moderate-severe heart failure symptoms (NYHA >2) at presentation in 29% of cases. Although cardiac symptoms were the reason that lead medical attention in all but two cases, a detailed neurologic evaluation at presentation disclosed a mild neuropathy (sensory abnormalities in the lower limbs) in 27% of cases. 39% of patients had a history of carpal tunnel syndrome.

Prevalence of atrial fibrillation was 30%, 6% first degree A-V block and 9% had a pacemaker implanted for bradiarrhythmia. Low voltages at ECG were present only in 17 patients (30%). On echocardiogram left ventricular diameters were normal (median end-diastolic diameter 48 [46-51] mm), with symmetric hypertrophy and a median interventricular septum thickness of 17 [16-20] mm (median voltage/mass ratio 0.5 [0.4-0.7] mV/gr/mq). Median left ventricle ejection fraction was 50 [42-61] %, a restrictive filling pattern was present in 36% of cases.

Among the 11 females, three patients had an exclusively neurologic phenotype at presentation, with both autonomic and a neurosensory involvement. Female patients with cardiac involvement had a similar ECG end echo profile compared to males.

Compared to patients with wt-TTR, Ile68 patients had a comparable cardiomyopathy, the only significant difference being age at diagnosis (71 [64-77] in Ile68 vs 78 [72-81] in wt-TTR, p<0.001).

Mean follow up was 24 months and during this period all patients were treated exclusively with non-disease modifying agents. Survival at 1, 3 and 5 years was 90%, 70% and 55%, respectively, slightly worse than age adjusted survival in wtTTR (p=0.189).

DISCUSSION & CONCLUSION: In this first detailed analysis of patients with Ile68 amyloidosis endemic in central-northern part of Italy, in particular in the Apennines Mountains the vast majority of patients had a prevalently cardiologic phenotype (exclusively cardiologic in 73%), and more than 80% were male. The echocardiographic phenotype consisted of a non-dilated left ventricle with concentric hypertrophy and a normal systolic function in 70% and a restrictive filling pattern in 30%. Overall, the clinical, ECG and echo phenotype did not differ from that of wt-TTR patients, with the exception of a younger age at diagnosis in Ile68.
Carotid artery stiffness is impaired in transthyretin cardiac amyloidosis

G Di Bella¹, A Mazzeo², R Costantino¹, D Di Nunzio¹, L Gentile², MP Campisi¹, C Stancanelli², M Casale¹, S Carerj¹, G Vita².

¹ Department of Neurosciences, University of Messina, Messina, Italy ² Department of Neurosciences, University of Messina, Messina, Italy.

INTRODUCTION: Familial amyloidotic polyneuropathy (FAP) is an autosomal dominant disease caused by a mutation of the gene coding for the transthyretin (TTR). Amyloid deposit can be observed in the cardiovascular system (both heart and vessels). Although many studies have investigated heart function, no data are investigated vascular artery function in CA. The aim of this study was to investigate the relationship between carotid artery stiffness in CA

MATERIAL & METHODS: seventeen normal subjects (Normals) and 9 CA patients on the basis of TTR gene mutation, left ventricular septum >15mm and typical cardiac magnetic resonance findings of cardiac amyloidosis and without conventional cardiovascular risks factors underwent carotid echo-tracking studies. Measurements of local arterial stiffness (pulse wave velocity [PWV, (m/s)], stiffness parameter, [β] and augmentation index [AI, %]) were obtained as a mean value from the right and left common carotid arteries about 1 cm proximal to the bulb region

RESULTS: No differences regarding age and gender were found between Normals (52 ± 9 years, 38%female) and CA (53 ±8 years, 36% female). Arterial stiffness was increased in the CA compared with the Controls (PWV: 12.1 ± 2.5 vs 6.3 ± 1.2, P<0.0001; β: 14.7 ± 5.4 vs 8.7 ± 3.7, P=0.003; AI: 28 ±12.8 vs 21.3 ± 12.8, not significantly but with a tendency, P=0.08).

DISCUSSION & CONCLUSIONS: Deposition of amyloidosis can be located in artery vessels. Carotid artery stiffness is impaired in TTR CA

REFERENCES:


PA89
Transthyretin-related amyloidosis in the Mediterranean and Balkan area: focus on the Glu89Gln mutation

A Milandri1, M Gospodinova2, A Mazzeo3, A Alfonsò1, S Sarafo4, S Ratti1, A Tinuper1, C Gagliardi1, S Foffi1, M M Cinelli1, M Lorenzini1, G Vita3, F Salvi2, C Rapezzi1, I Tournev4

1 Cardiology, Department of Experimental, Diagnostic and Specialty Medicine – DIMES, University of Bologna, Italy. 2 University Hospital Alexandrovska, Clinic of Cardiology, Sofia, Bulgaria. 3 Department of Neurosciences, Messina University, Messina, Italy. 4 University Hospital Alexandrovska, Clinic of Neurology, Sofia, Bulgaria. 5 Bellaria Hospital, Neurology, Bologna, Italy.

Agnesemilandri@hotmail.it

INTRODUCTION: Glu89Gln is one of the most frequent transthyretin (TTR) mutations in the Mediterranean and Balkan areas and has been associated with a cardiologic, neurologic and mixed phenotypes. Even though this variant has been described worldwide, it remains to be established whether geographical area influences the phenotypic disease expression. Even though case reports have been previously published, a detailed phenotype analysis, comparison of the patients from the two geographic areas and outcome data are still not available.

MATERIAL & METHODS: We retrospectively analysed all consecutive subjects with Gl89Gln transthyretin mutation evaluated at two Italian centres (Bologna and Messina) and one Bulgarian centre (Sofia) between January 1991 and December 2015. Of the 109 subjects with the mutation (69 from Italy and 40 from Bulgaria), from 54 unrelated families, 86 patients were affected (46 from Italy and 40 from Bulgaria) and 23 were asymptomatic carriers. We analysed baseline clinical, ECG and echocardiographic findings of the affected patients.

RESULTS: A preliminary comparison between Italian and Bulgarian cohort showed similar clinical, ECG and echo findings in both series. The only exception is the more frequent presentation with neurological symptoms in Bulgaria, that can be explained by the specificity of the local neurological centre. Disease penetrance was 50% at 55 years old in both centres. Median age at diagnosis was 56 [52-60], male prevalence was 59%. The majority of patients (76%) had a mixed phenotype at presentation (43% with neurological involvement earliest). Isolated cardiac and neurologic involvement were present in 10% and 13%, respectively. More than half of patients had a history of carpal tunnel syndrome. Neurologic involvement was characterized by a peripheral polineuropathy, that was associated with an autonomic involvement in most cases. Fourteen patients (18%) had a normal ECG at presentation. The most frequent ECG abnormalities were pseudo-infarction (53%), low QRS voltages (35%), left anterior hemiblock (34%) and negative T waves (18%). Only 6 patients showed atrial fibrillation at presentation (8%) and 4 (5%) had previously received a pacemaker. Echocardiography revealed concentric hypertrophy with a median diastolic interventricular septum thickness of 18 [15-20] mm and normal left ventricle diameters (end-diastolic diameter 45 [41-49] mm). Median left ventricular ejection fraction (LVEF) was 61 [52-66]% and only 11 patients (15%) had a LVEF < 50%. A restrictive filling pattern was present in 31 patients (41%). No differences were present comparing affected males and females, with the exception of age at diagnosis that was higher in the female group (females 58 [55-63] vs males 53 [49-56], p<0.001).

DISCUSSION & CONCLUSIONS: This is the largest series of patients with Glu89Gln TTR-related amyloidosis analysed in detail. The mutation appears to be endemic in southern Italy and Bulgaria. Clinical phenotype is typically mixed (neurologic and cardiac) with concentric left ventricular hypertrophy, non-dilated left ventricular diameters and normal ejection fraction. Survival curves at 5 years demonstrate an unfavourable prognosis.
Cardiac conduction abnormalities in 154 patients with hereditary transthyretin amyloidosis in Spain

E González-López1,2, Amor-Salamanca A1, J. González-Costello3, J. Pons Linares4, F. Muñoz-Beamud5,

S. Azorín6, JJ. Vílchez Padilla7, J. Buades8, L. Galán8, F. Martinez-Valle10, J. Fernández Martín11,
P. García-Pavía1,2

1Heart failure and inherited cardiac disease Unit. Department of Cardiology. Hospital Puerta de Hierro Majadahonda, Madrid, Spain. 2Department of Molecular Regulation of Heart Failure, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid. 3Advanced Heart failure and Transplant Unit, Hospital Universitario de Bellvitge, L’Hospitalet de Llobregat, Barcelona. 4Cardiology Department, Hospital Son Espases, Palma de Mallorca. 5Internal Medicine Department, Hospital Juan Ramón Jiménez, Huelva. 6Nephrology and transplant Unit, Hospital Clínico de Barcelona, Barcelona. 7Neurology Department, Hospital Universitario y Politécnico La Fe, Valencia. 8Internal Medicine Department, Hospital Son Llatzer, Palma de Mallorca. 9Neurology Department, Hospital Clínico San Carlos, Madrid. 10Internal Medicine Department, Hospital Vall d’Hebrón, Barcelona. 11Internal Medicine Department, Hospital Meixoeiro, Vigo, Spain.

esthgonzalez@hotmail.com

INTRODUCTION: Conduction abnormalities have been reported to be frequent in hereditary transthyretin amyloidosis (mATTR). Their management in daily clinical practice remains challenging1. There is conflicting evidence among different published series and there is no consensus regarding the optimal timing to implant a prophylactic pacemaker (PM) in mATTR1. The objective of this study was to assess the incidence/evolution of conduction abnormalities and determine PM implantation rate in mATTR in Spain.

MATERIAL & METHODS: We conducted a retrospective and observational study, including 10 mATTR centres in Spain. ECG characteristics were assessed according to standard definitions 2,3 and first/last ECGs were evaluated when available. Cardiac involvement was considered in the presence of 1) unexplained left ventricular hypertrophy (LVH) >12mm on echocardiogram, 2) intense uptake on 99mTc-DPD scintigraphy (Perugini score 2-3) and 3) conduction abnormalities requiring PM implantation.

RESULTS: A total of 154 patients (46±16 years at diagnosis; 60% males) were studied. The predominant TTR mutation was Val30Met (121 patients, 79%), followed by Glu89Lys and delV122 (5%, each). 74 (48%) patients had undergone liver transplant, 6 (4%) had received a cardiac transplant and 36 (23%) were asymptomatic genetic carriers. Among the 117 patients (77%) with neurological symptoms, the vast majority were in Coutinho stage I (72 patients, 64%). 72 patients (47%) were considered to have cardiac involvement, among whom, mean maximal LV wall thickness was 15±4 mm. Only 11 patients (7%) showed atrial fibrillation (AF). Among the whole cohort, 25 patients (17%) had a PM implanted. The most frequent indication for PM implantation was arrhythmia prophylaxis (12 patients, 48%), followed by complete (6, 24%) and second-degree atrioventricular block (3, 12%). Among the 102 patients (66%) with 2 ECGs available, we observed significant progression of PR interval (169±31 vs. 181±41 ms; p=0.001), widening of QRS (92±20 vs. 98±23 ms; p=0.004) and prolongation of QTc (426±36 vs. 436±37 ms; p=0.008) after a median of 2 (IQR 1-7) years. No significant differences were observed in the development of low voltage, pseudo-infarct pattern, LVH or conduction abnormalities. However, genetic carriers were more likely to develop left anterior fascicular block and pseudo-infarct pattern during follow-up (p=0.03 and p=0.005, respectively).

DISCUSSION & CONCLUSIONS: Cardiac involvement is frequent in mATTR in Spain. Conduction abnormalities were frequently found but AF was uncommon. Although a significant prolongation of ECG intervals over time was noticed and genetic carriers develop electrocardiographic abnormalities during follow-up, the rate of clinically indicated PM implantation in our series was low.

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Doxycycline and TUDCA combination slow the progression of ATTR amyloid cardiomyopathy in humans.

T Mirto, D Dupee, H Geller, RH Falk

Brigham and Women’s Hospital Cardiac Amyloidosis Program, Boston MA, USA
rfalk@partners.org

INTRODUCTION: The combination of doxycycline and tauroursodeoxycholic acid (TUDCA) has been shown to reduce amyloid fibrillogenesis and to prevent intracellular toxic prefibrillar deposits in a transgenic mouse model of familial amyloidosis. The effect of this drug combination is not known in humans with ATTR cardiomyopathy, and we therefore undertook a study of doxycycline and TUDCA to determine whether these agents, administered together, show any effect of disease progression in patients with ATTR cardiomyopathy.

MATERIAL & METHODS: 40 patients with TTR cardiac amyloidosis were enrolled. Doxycycline was prescribed at 100 mg twice daily, and TUDCA 250 mg three times daily. Patients had an echocardiogram at baseline, and at 6, 12 and 18 months of therapy, and routine lab work, NTproBNP and troponin were measured. The primary endpoint was change in longitudinal strain between baseline and 12 months. Comparison was made to an historical control group of TTR amyloidosis patients of similar status, who had had no specific treatment. This report details preliminary results in a group of patients reaching the 12 month study point.

RESULTS: 30 treatment-group patients, mean age 71.3 yr (1 woman) had an enrollment period >12 months from study entry. All but 3 had ATTRwt. 3 patients died within this period, 2 had technically inadequate follow-up studies, 1 had a pacemaker inserted after the baseline echo and 2 were unable to tolerate therapy. Mean strain at baseline was -11.5 +/-2.9% in the control group and -10.8 +/- 2.3% in the treated group, (p=ns). At a mean follow-up of 12 months, both groups showed a deterioration in LV strain: Strain in control patients fell from -11.5 to -8.5% (p <0.004) and strain in treated patients from-10.8 to -9.8% (p<0.02). A comparison of the percentage change in individual patients showed that control patients had a greater deterioration in cardiac function than treated patients: p=0.006 (Figure). NT-proBNP was not measured in the historical control group, but in the treatment group, it rose slightly, but significantly, from a mean of 3108 pg/ml to 3669 pg/ml (p <0.05).

CONCLUSIONS: Doxycycline –TUDCA combination was generally well-tolerated in patients with ATTR cardiomyopathy. Despite therapy, cardiac longitudinal contraction deteriorated slightly over a 1-year period, associated with a mild rise in NTproBNP. Thus the combination does not prevent disease progression. However, when compared with an historic control group, disease progression in treated patients was slower than expected. These promising results suggest that a randomized, placebo-controlled study may be warranted and would be feasible to perform.
PA92

Looking into genetic variation within the TTR gene: functional impact of regulatory SNPs in FAP ATTRV30M

M. Alves-Ferreira¹,2, A. Azevedo¹,2, T. Coelho³, D. Santos¹,2, J. Sequeiros¹,2, I. Alonso¹, A. Sousa¹,2, C. Lemos¹,2

¹ UnIGENe, IBMC—i3S, Porto, Portugal; ² ICBAS -Instituto Ciências Biomédicas Abel Salazar, University of Porto, Porto, Portugal, ³ Unidade Corino de Andrade (UCA), Centro Hospitalar do Porto (CHP), Porto, Portugal

miguel.ferreira@ibmc.up.pt

INTRODUCTION: Familial amyloid polyneuropathy (FAP) is an autosomal dominant neurodegenerative disease with onset on adult age that is characterized by mutated protein deposition in the form of amyloid substance. FAP is due to a point alteration in the transthyretin (TTR) gene and until now more than 100 amyloidogenic mutations have been described in TTR gene. V30M mutation does not explain by itself the symptoms and age-at-onset variability of the disease observed in the same family.

OBJECTIVE: Our aim was to identify genetic factors associated with phenotypic variability and reduced penetrance which can have important clinical implications.

MATERIAL & METHODS: To accomplish this we genotyped 326 FAP Portuguese patients, using a direct-automated sequencing approach of the exons and flanking regions of the TTR gene. After genotyping an intensive in silico analysis was performed in order to understand a possible regulation of gene expression.

RESULTS: We identified three previously undescribed polymorphisms and also very interesting and unreported results in the in silico analysis as we found some polymorphisms leading to alterations in the mechanism of splicing, transcription factors binding sites and miRNAs binding.

DISCUSSION & CONCLUSIONS: All these mechanisms when altered can lead to dysregulation of TTR expression, which can have an impact in age-at-onset and phenotypic variability. These putative mechanisms of gene expression regulation within the TTR gene could be used in the future as potential therapeutical targets, and could improve genetic counselling and follow-up of mutation carriers.
Four-year progression of small fiber neuropathy quantified with Quantitative Sensory Testing (QST) in patients with Familial Amyloid Polyneuropathy (FAP)

O. Carranza¹, K. Cárdenas¹, K. García¹, C. Bañuelos¹, C. Domínguez¹, A. González Duarte¹

¹Department of Neurology National Institute of Medical Sciences and Nutrition “Salvador Zubirán” Mexico City, Mexico.

octavio.carranza.r@gmail.com

INTRODUCTION: Small fiber neuropathy is one of the earliest and most common symptoms in FAP. Quantitative sensory testing is a novel method to detect changes in the perception of thermal and vibratory stimuli. When performed regularly, it can detect small changes in perception, which may indicate disease progression.

MATERIAL & METHODS: 58 patients with FAP were evaluated using the method of limits with a TSA-II neurosensory analyzer (Medoc®). Cold and warm detection thresholds were measured first (CDT, WDT) and then cold pain and heat pain were determined (CP, HP). Subjects indicated when the stimulus was initially felt or painful and the operator stopped the stimuli. The mean threshold temperature of three consecutive measurements was calculated. The baseline temperature was 32°C with cut-off temperatures form 0 to 50°C. Vibratory stimulus was then applied in the third finger and first toe.

RESULTS: Temperature, pain and vibration thresholds were compared at baseline (n=58), 1 year (n=28), 2 years (n=13) and 4 years (n=2). At baseline, mean CDH was 25.4±7.6°C in hands, and 22.9±8.31°C in feet; mean WDH was 37.2±4.7°C in hands and 40±4.7°C in feet; mean vibration threshold was 4.4±12 in hands and 12.3±24 in feet. When comparing to assessments performed three years later, there was a change of +0.4°C in WDT in hands, of -0.9°C in CDT in feet; of +0.8°C in CP in hands, and of +2.2°C in feet. When comparing baseline to the 4th year evaluation, there was a change in CDT of -10.6°C in hands and of -10.4 in feet; WDT changed +5.6°C in hands and +8°C in feet; CP changed -8.9°Cin hands and -12.1°C in feet; WP changed +4.8°C in hands and +4.6 in feet; and vibratory thresholds changed 1.7Hz in hands and 0.2Hz in feet.

DISCUSSION & CONCLUSION: Initial evaluation showed important changes in thresholds when compared to baseline (-7.8°C lower from the baseline in CDT and +6.6°C higher in WDT). Changes were more pronounced after the fourth year, where a rapid decline of function in all modalities occurred in two patients. The most sensible threshold was CP, meaning that patients developed pain at higher temperatures (they were more sensitive to cold). QST is a useful and easy technique to detect clinical progression of loss of small nerve function in subjects with FAP. This is important because the earliest detection of disease is needed to begin modifying treatment in carriers of hereditary FAP.

Natural history of Ser50Arg mutation in a Mexican population

K. Cárdenas1, O. Carranza1, K. García1, C. Bañuelos1, A. González Duarte1.

1Department of Neurology National Institute of Medical Sciences and Nutrition “Salvador Zubirán” Mexico City, Mexico.

cask_27@hotmail.com

INTRODUCTION: Ser50Arg is a very rare mutation associated with Familial Amyloid Polyneuropathy. Large cohorts of patients with this mutation have not been described.

MATERIAL & METHODS: All patients with Familial Amyloid Polyneuropathy or first degree family members with the mutation seen at the National Institute of Medical Sciences and Nutrition during a four-year period with a Gene Sequence Analysis of TTR positive for a TTR mutation underwent a thorough examination that was noted in a database which included the state of the patient at the time of diagnosis, age, sex, birth place, initial symptoms, and current clinical condition of the patient. Patients were followed at a regular basis in our clinic and submitted to Quantitative Sensory Testing, Quantitative Autonomic Testing and SUDOSCAN for evaluation of the progression of the disease.

RESULTS: Of 108 patients registered at our database with TTR Amyloidosis, we found that 85 (78.7%) were positive for the Ser50Arg mutation. Of them 43 (50.58 %) were male and 42 (49.41%) were female. There are approximately 22 affected families. At the time of diagnosis 63.52% of the patients were symptomatic. Mean age at onset of symptoms was 36 years old. The initial symptoms were: neuropathic 47.05%, gastrointestinal 10.58%, cardiovascular 2.35%, genitourinary 1.17% and autonomic 1.17%. Neupathy Dissability Score was obtained at the time of diagnosis: 56.47% were at stage 0, 15.29% were at stage I, 11.76% were at IIIA, 1.17% at IIIB and 8.23% at stage IV. Of the 40 patients with clinical neuropathy at onset, 17 were men at mean age 39.5 years and 23 were women at mean age 39 years. Neuropathy score progressed in 15.29% of the patients in 18 months time from 0 to I in 2.35% patients, from I to II in 5.88%, from II-III in 3.52%, from II-IIIB 1.17% and from IIIA to IV 2.35%. Six patients (7.05%) required a pacemaker in 2.5 years mean time from symptom’s onset due to arrhythmias. None of the patients developed albuminuria. Eleven patients (12.94%) died at a mean age of 44 years of age. Three patients (3.5%) had a liver transplant, however neuropathy and cardiopathy also progressed in these patients.

DISCUSSION & CONCLUSION: We confirmed that in the Mexican population Ser50Arg is the most prevalent mutation with an age of onset around 36 years of age and neuropathic initial symptoms. Neuropathic progression and cardiovascular progression was frequent in almost one third of the patients, despite liver transplantation.

Epidemiological and clinical characteristics of persons with transthyretin familial amyloid polyneuropathy: A global synthesis of 532 cases

M Waddington Cruz1, H Schmidt2, MF Botteman3, JA Carter4, AS Chopra3, M Stewart5, M Hopps5, S Fallet5, L Amass5

1 Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. 2 Universitätsklinikum Münster, Münster, Germany. 3 Pharmerit International, Bethesda, MD, USA. 4 BluePoint, LLC, Chicago, IL, USA. 5 Pfizer Inc, New York, NY, USA.

mwaddingtoncruz@gmail.com

INTRODUCTION: Transthyretin familial amyloid polyneuropathy (TTR-FAP) was first identified in Portugal in 1952 [1]. In 1984, the first report of a causative mutation – substitution of methionine for valine in the transthyretin gene (Val30Met) – was published [2]. Since then approximately 100 causative mutations have been identified [3], which have likely driven the phenotypic variability of this rare disease. The purpose of the present study was to assess the epidemiological and clinical variability of TTR-FAP by synthesizing evidence extracted from published case reports.

MATERIAL & METHODS: Electronic searches applied to four databases (EMBASE, PubMed, SCOPUS, and Web of Science; all languages included) identified individual TTR-FAP cases from case reports, case series, and cohort studies published from 2005-2015. Manual review of presentations and abstracts from four annual clinical conferences (ISA 2010, 2012, 2014 and ECHATTR 2015) was also conducted. Data for the following variables, for each patient, were collected: (A) confirmation of symptomatic disease manifested by polyneuropathy, (B) gender, (C) mutation, and (D) location/country. These variables constituted the minimum threshold for retention in the database as a “case”. The following variables were also extracted where found: ages of (E) symptom onset, (F) diagnosis, and (G) death, symptoms at (H) onset and (I) diagnosis, and (J) parent-of-origin effect (POE; genotypically confirmed). Duplicate cases were identified by overlap of variables B-I, and subsequently removed. Cases of de novo disease subsequent to liver transplantation were excluded. Analysis included descriptive interrogation of variables E-J stratified by variables B-D and multinomial logistic regression to assess the extent to which clinical and demographic variables predict mutation.

RESULTS: The search strategy yielded 3,003 records from which N=532 unique cases were identified, distributed across 30 countries, with Japan being the most frequent (18.6%). Val30Met was the most common mutation observed (48.9%) followed by Ser77Tyr (13.2%). Cases were predominantly male (67.5%). The mean (standard deviation) ages of onset and diagnosis were 55.0 (±14.0) years and 61.4 (±12.7) years, respectively. Among cases with ages of onset and death reported (n=126), the mean duration from onset to death was 6.3 years. Symptomatology consisted primarily of progressive lower limb sensory impairment. Temporal variables appeared to be most predictive of the mutational profile. Genotypically-confirmed POE was not reported in sufficient detail for analysis.

DISCUSSION & CONCLUSIONS: TTR-FAP is a rare and diverse disease, with phenotypic variability likely driven by the occurrence of various non-Val30Met mutations in non-endemic countries and Japan. Given the importance of early diagnosis and treatment on the disease progression, increasing awareness among providers and facilitating specialized treatment access for patients may markedly improve long-term outcomes.

A phase II study of doxycycline plus tauroursodeoxycholic acid in transthyretin amyloidosis

L. Obici1, S. Perlini2, G. Palladini1, A. Cortese1, P. Milani1, R. Mussinelli2, F. Salinaro2, A. Lozza3, E. Alfonsi3, M. J. Saraiva4, G. Merlini1

1Amyloidosis Research and Treatment Center, Foundation IRCCS Policlinico San Matteo, and Department of Molecular Medicine, University of Pavia, Pavia, Italy. 2Clinica Medica II, Foundation IRCCS Policlinico San Matteo, and Department of Internal Medicine, University of Pavia, Pavia, Italy. 3General Neurology and Neurophysiopathology Unit, Foundation IRCCS C. Mondino National Institute of Neurology, Pavia, Italy. 4Grupo de Neurobiologia Molecular, IBMC, Universidade do Porto, Porto, Portugal.

l.obici@smatteo.pv.it

INTRODUCTION: Several in vitro and in vivo observations support the anti-amyloidogenic activity of tetracyclines. Treatment with doxycycline and tauroursodeoxycholic acid (TUDCA) has shown a synergistic effect on removal of TTR deposits in the mouse model of FAP. The safety and tolerability profiles of doxycycline and TUDCA are well established and favourable. We conducted a phase II, open-label study to evaluate the efficacy, safety and pharmacokinetics of oral doxycycline (100 mg BID) and TUDCA (250 mg three times/day) in patients with TTR amyloidosis.

MATERIAL & METHODS: Treatment was administered daily for 12 months. Primary endpoint was response rate at 12 months defined as: < 2 point increase in NIS-LL in patients with neuropathy and less than 30% or < 300 pg/mL increase in NT-proBNP concentration in subjects with cardiomyopathy. Entry criteria included symptomatic disease due to hereditary or senile ATTR. Patients with a progressive disease 1 year after liver transplantation were eligible. Evaluations were performed at entry, 6 and 12 months. A follow-up assessment was scheduled six months after treatment completion (M18).

RESULTS: 40 subjects (30 males, median age 68 years) were enrolled. 25 patients have hereditary ATTR (Val30Met in 7), 13 have wild-type ATTR and 2 were domino-transplanted. 5/25 patients underwent previous liver or liver/heart transplantation. 20 patients (50%) had PNS involvement with median (range) NIS-LL being 31 (2-70). Cardiac amyloidosis was present in 35 patients (88%). Median mLVW was 16.1 mm (11.7-20.6), NT-proBNP 1684 ng/L (407-10771). 14 patients discontinued treatment before M12 visit (4 due to GI events, 2 for rash at limbs, 2 for profound weakness, 1 for neurological disease progression, 1 due to indication to tafamidis, 1 breast cancer and 3 voluntarily discontinued). Treatment was well tolerated in all the other patients, except for mild redness at hands and face. 26 patients completed the 12-month treatment period. 13 were evaluable for neurological disease. Stable neuropathy (NIS-LL < 2) was observed in 6/13 patients. PND remained unchanged in all. 25 patients were evaluable for heart disease. A stable disease according to NT-proBNP changes was observed in 17/25 (68%) patients. BNP was stable in 20/25 (80%) patients. 21 patients were evaluated six months after treatment was completed. 11 (52%) had progressed according to BNP. The rate of BNP progression after treatment discontinuation was significantly higher (p=0.002) compared to treatment period. Twelve-month DOXY-TUDCA treatment was associated with improved global left ventricular longitudinal strain (GLS (mean); from -9.75±0.51% to -12.99±0.47%, p<0.05), an index of systolic function. All other echo-derived indices of diastolic and systolic function did not change during treatment. Interestingly, during the 6-month washout period worsening in both right ventricular systolic function (as assessed by tricuspid annulus systolic excursion (mean); from 17±2 to 13±3 mm, p<0.05) and GLS (from -12.99±0.47% to -10.87±0.32%, p<0.05) was observed. Quality of life and mBMI were maintained.

DISCUSSION & CONCLUSIONS: Although a higher than expected dropout rate was observed, our study indicates that treatment with doxycycline and TUDCA may be associated with slowing of cardiac disease progression in a subset of patients.
Val122Ile TTR amyloid cardiomyopathy in African-American patients has a worse prognosis than wild-type TTR amyloid cardiomyopathy

TM Mirto, HI Geller, A Singh, D Dupee, RH Falk

Brigham and Women’s Hospital Cardiac Amyloidosis Program, Boston MA, USA
tmirto@partners.org

INTRODUCTION: Transthyretin Val122Ile, is found in 3.8% of the African-American population. The degree of penetrance is unknown, but the gene is associated with an increased risk of heart failure, regardless of presence of typical amyloid phenotype. There are conflicting data regarding survival in Val122Ile versus wild-type TTR cardiac amyloidosis (ATTRwt), with 1 recent international multicenter database study (1) suggesting similar outcomes. It is important to know the natural history, since new drugs may slow all forms of TTR amyloidosis, and trials of new agents are enrolling both ATTRwt and ATTRm, including Val122Ile. We therefore evaluated all patients seen with typical Val122Ile amyloid cardiomyopathy in a single center, and compared outcome to a group of ATTRwt.

MATERIAL & METHODS: 35 Val122Ile patients (10 female) with amyloid cardiomyopathy were seen over an 8 year period and compared to records of 35 randomly selected patients with ATTRwt (2 female). Onset of cardiac symptoms likely due to amyloidosis was estimated. Date of diagnosis was based on cardiac biopsy or (for Val122Ile) either biopsy or typical echo with positive genetic testing. Death was determined by national database of deaths.

RESULTS: Mean age at diagnosis did not differ between groups: Val122Ile =71.4 yr, SSA =73.9 yr. There was a significant difference in sex distribution between Val122Ile (28% female) versus ATTRwt (6% female, p <0.01), consistent with the known male prevalence of ATTRwt. Median actuarial survival from diagnosis differed statistically between groups, being 49 months in Val122Ile and 70 months in SSA (p<0.05) (Fig 1). To determine whether delayed diagnosis in Val122Ile was responsible for the worse prognosis, we evaluated time from first symptoms in each group. Among those in whom initial symptoms could be determined (31 Val122Ile and 32 ATTRwt) there was a trend toward earlier diagnosis in Val122Ile: mean time to diagnosis was 21.6 months in Val122Ile and 32.4 months in ATTRwt and (P=NS).

DISCUSSION & CONCLUSIONS: 1. Contrary to previous suggestions, patients with Val122Ile amyloid cardiomyopathy have a significantly worse prognosis from diagnosis than do patients with ATTRwt cardiomyopathy. 2. This difference cannot be explained by a delayed diagnosis in the Val122Ile patients, as they had a trend toward earlier diagnosis. 3. These findings underscore the poorer prognosis in Val122Ile compared to ATTRwt amyloid cardiomyopathy, despite the fact that both diseases are due to cardiac infiltration with TTR-derived amyloid and possibly suggest a faster deposition in Val122Ile. 4. The poor prognosis in Val122Ile, a disease to which 3.8% of the African-American/Afro-Caribbean population is predisposed, underscores the pressing need for high clinical suspicion of amyloidosis when heart failure occurs in this population.


Fig. 1: Val122Ile survival versus ATTRwt
STANDARD HEART FAILURE MEDICATION IN CARDIAC TRANSTHYRETIN AMYLOIDOSIS: USEFUL OR HARMFUL?

Fabian aus dem Siepen, S Hein, R Bauer, H A Katus, A V Kristen

1 Department of Cardiology, University Hospital Heidelberg, Heidelberg, Germany

Fabian.Siepen@med.uni-heidelberg.de

INTRODUCTION:
Guidelines suggest the use of ACE inhibitors (ACEi), beta-blockers and diuretics in all patients with symptomatic heart failure, independent of the aetiology. While diuretics are able to reduce symptoms, the use of ACEi and beta-blockers in cardiac transthyretin amyloidosis (ATTR) has not been elucidated in detail. We sought to investigate the outcome of ATTR patients on different combinations of medication retrospectively.

MATERIAL & METHODS:
Medical records of 480 patients (wtATTR: n=242, mATTR: n=238, n= 72 Val30Met, n=7 Val122Ile, n=63 Val20Ile, n=96 other mutations) were screened for long-term medication as well as clinical, laboratory, electrocardiographic and echocardiographic data. Patients were assessed between 2001 and 2015 at the University Hospital Heidelberg. Mean follow-up was 41±4 months.

RESULTS:
257 (54%) patients were treated with ACEi (wt:ATTR: 176 patients/72%, mATTR: 81 patients/34%) and 239 (50%) patients with beta-blockers (wtATTR: 162 patients/68%, mATTR: 77 patients/32%). Characteristics of mATTR patients are given in Table 1. In mATTR amyloidosis, survival was significantly shorter in patients treated with beta-blockers or ACEi (figure 1). In wtATTR no significant differences were observed between patients with/without ACEi or betablocker regarding clinical parameters and survival (ACEi vs. non-ACEi p=0.4; betablockers vs. non-betablockers p=0.7).

DISCUSSION & CONCLUSIONS:
In mATTR, survival was significantly better among patients not receiving standard heart failure medication, suggesting a potential disadvantage of ACEi and betablockers, even though patients on heart failure medication had a more severe cardiac involvement. Furthermore, we demonstrated that patients with wtATTR did not benefit from treatment with ACEi and beta-blockers. These findings need to be investigated further, ideally in a blinded, prospective study.

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Table. 1: Patient characteristics of mATTR patients with and without heart failure medication

Fig. 1: Kaplan-Meier analysis of patients with mATTR with and without heart failure medication
Ruptured distal biceps tendon: a new non-cardiac clinical sign of wild-type cardiac amyloidosis

HI Geller, TM Mirto, A Singh, D Dupee, RH Falk

Brigham and Women's Hospital Cardiac Amyloidosis Program, Boston MA, USA higeller@partners.org

INTRODUCTION AND METHODS: Wild-type TTR cardiac amyloidosis (ATTRwt) predominantly affects elderly men, causing progressive heart failure (CHF). It is often associated with carpal tunnel syndrome (CTS), which can predate CHF by 5-10 years (1). Histologic examination at the time of surgery for CTS, rotator cuff tears and spinal stenosis in a group of patients with a mean age of 65, showed evidence of TTRwt amyloid deposits in the flexor tenosynovium in 18/54 patients (33%), rotator cuff in 5/21 (24%) and in yellow ligaments in 16/36 (44%), yet none had evidence of systemic amyloidosis (2). Spontaneous rupture of the distal biceps tendon (RBT) is relatively uncommon condition, with an estimated incidence of 2-7 per 100,000 person years (3). It is characterized by the appearance of a “Popeye” bunching of the biceps muscle on elbow flexion, and failure of the biceps to move with supination to pronation of the wrist (4). Having noticed a unilateral “Popeye” biceps in several patients with biopsy-proven ATTRwt cardiac amyloidosis, we systematically questioned TTR amyloid patients about symptom of CTS or RBT, and examined them for the presence of RBT, seeking information about possible date of onset.

RESULTS: Over 25 months, 111 patients were seen with cardiac ATTRwt. Mean age was 74.9 ± 6 years and all but 3 were men. 55/111 (49.5%) had a history of CTS and 37 (33.3%) had clinical evidence of RBT. RBT occurred in the dominant limb in 35/37 patients and was bilateral in 9. Of 39 patients able to estimate the onset of CTS symptoms, these occurred a mean of 9.9 years before CHF. 14 were unaware of their RBT or did not know the onset. In the remainder, estimated onset was an average of 7.3 years before CHF. There was a strong correlation between the presence of RBT and CTS, which occurred concurrently in 22.5% of patients, 25/37 of those with RBT also having CTS (p=0.009 by Fisher’s exact test).

DISCUSSION & CONCLUSIONS: The prevalence of RBT in our patients with transthyretin amyloidosis is >1000 times higher than expected by chance, and strongly suggests a causal factor of TTR tendon deposits. Early diagnosis of TTR amyloidosis is important now that potential therapies are in clinical trials and RBT is a simple diagnostic sign test which, when present, suggests increased likelihood that an elderly man presenting with heart failure has ATTRwt amyloidosis.

REFERENCES:

Fig. 1A: Acute spontaneous biceps tendon rupture in a 77 year-old man with biopsy-proven ATTRwt cardiac amyloidosis. This occurred 5 years after cardiac diagnosis 1B. “Popeye” sign of rupture biceps tendon, present at diagnosis of ATTRwt cardiac amyloidosis in a 76 year old man
PA100

Metabolomic analysis for diagnosis and biomarker discovery of transthyretin amyloidosis: A pilot study

Malin Olsson, Jonas Wixner and Intissar Anan
Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

Background

Metabolomics is a quantitative measurement of the multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modifications, which is often used in toxicity assessment, disease diagnosis, and functional genomics. By measuring changes in metabolite concentrations, the range of biochemical effects that are induced by a disease can be determined.

In this study, we aimed to characterize the metabolomic profile of transthyretin amyloid (ATTR) amyloidosis and to identify potential diagnostic biomarkers for the disease.

Material and methods

Twenty-seven ATTR V30M amyloidosis patients, 27 gene carriers of the TTR V30M mutation with no evidence of the disease, and 26 healthy controls were included in the study. Low molecular weight components in the plasma was analysed by Gas Chromatography-Mass Spectrometry (GC-TOF-MS) and Liquid Chromatography-Mass Spectrometry (LC-QTOF-MS).

Results

The results of the pilot study are partially concluded and the preliminary results show that patients diverge from healthy TTR V30M carriers and controls with respect to the metabolic fingerprint, Figure 2.

Discussion and conclusions

The biomarkers identified revealed that pathophysiological changes in ATTR could help to discriminate between ATTR amyloidosis patients and healthy controls. The predictability of this method suggests its potential application in the diagnosis of ATTR.
PA101

Effect of doxycycline and ursodeoxycholic acid on transthyretin amyloidosis

Jonas Wixner, Björn Pilebro, Hans-Erik Lundgren, Malin Olsson and Intissar Anan
Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

Background

Doxycycline has been shown to disrupt transthyretin amyloid (ATTR) fibrils [Cardoso et al 2006] and tauro-ursodeoxycholic acid (TUDCA) has been shown to reduce TTR toxic aggregates in mice [Macedo et al 2008]. Further, in 2010 Cardoso et al showed that a combined doxycycline/TUDCA treatment has a synergistic effect on ATTR deposition. Ursodeoxycholic acid (UDCA) is a bile acid used for the treatment of certain cholestatic syndromes with an efficacy similar to that of TUDCA. Based on this knowledge, we wanted to explore if treatment with doxycycline and UDCA (Dox/Urso) would prevent disease progression in ATTR amyloidosis. UDCA was selected since TUDCA is not available in Sweden.

Materials and methods

The Dox/Urso study was a phase II, 18-month prospective study on patients with ATTR cardiomyopathy divided into two parts. Primary end-points were changes in s-pro-BNP and total Kumamoto score from baseline. Part I was a 12-month, open label treatment period with doxycycline 200 mg/day for 4 weeks, with intermittent discontinuation for 2 weeks, and UDCA 750 mg/day. Part II was a withdrawal period during which disease progression was monitored. During part I, patients were evaluated at baseline and after 6 and 12 months of treatment. During part II, the patients were reassessed at month 18. Monthly phone contacts and blood tests (complete blood count, creatinine, aspartate transaminase, alkaline phosphatase and bilirubin) at 1, 3, 6, 9, 12 and 18 months were performed as safety measures.

Results

Twenty-eight patients were included of which one had wild-type ATTR amyloidosis and the others carried TTR mutations (twenty-one V30M, four H88R, one A45S and one A97S). Only 14 % had completed the whole study, whereas sixty-four % had completed 6 months and 36 % 12 months of treatment. The main reasons for early termination were treatment failure (14 %), which was pre-defined as an increase in Pro-BNP > 30% from baseline, side effects from the study drug (14%), consent withdrawal (24 %), denial to complete the withdrawal period (14%) and death (7 %). There were no changes in pro-BNP after 6 months of treatment (mean 6120 vs. 6137 ng/l, p = 0.97) but after 12 months it had increased significantly (mean 7463 vs. 9341 ng/l, p < 0.01). Cardiac septum thickness did not increase significantly during 12 months of treatment (mean 13 vs. 15 mm, p = 0.7). Total Kumamoto score remained stable over the first 6 months (mean 23 vs. 23,5 points, p = 0.68), but increased after 12 months (mean 25 vs. 31 points, p < 0.01). The nutritional status remained stable during 12 months (mean mBMI 926 vs. 897, p = 0.3). No significant changes were found in the safety follow-up blood tests.

Discussion and conclusions

The present study is flawed by the extreme high dropout rate that was caused by treatment failure, side effects and voluntary drop-outs, which may indicate lack of efficacy of the treatment. Also for the patients that could be evaluated, deterioration was noted both regarding heart function measured by pro-BNP and the overall condition measured by the Kumamoto score. For the latter, a deterioration of six points was noted, a figure similar to that obtained in the controlled trial of diflunisal (Berk J et al. JAMA 2013). Obici et al showed disease stabilization in treated patient up to 12 months with continuously administered doxycycline and TUDCA, which indicates that the 2 weeks intermittent discontinuation of doxycycline may substantially decreasing the efficacy of the drug, and that TUDCA may be more effective than UDCA. Further studies are needed to evaluate the long-term effect of combination treatment with UDCA/TUDCA/Doxycycline.
PA102

The role of gender and onset age as predictors of ophthalmologic changes in transthyretin familial amyloid polyneuropathy (TTR-FAP) patients

Introduction

To evaluate predictors of specific ocular changes in TTR-FAP patients on tafamidis

Methods

We performed a retrospective analysis of 129 patients transthyretin familial amyloid polyneuropathy (TTR-FAP) disease, on tafamidis, to study the influence of gender, the onset age, as well as the evolution time of the disease (years) on the occurrence of several ophthalmological events (amyloid deposits on lens anterior capsule, amyloid deposits on pupillary border, scalloped pupil, and vitreous opacities). The analyses were done using proportional hazards Cox multivariable regression models. The follow-up time was calculated since the first ophthalmologic evaluation until February, 2016.

Results

Sixty-eight patients were female (53%). Mean onset age was 39±13 years, the mean evolution time of the disease was 1.8±9 years at the beginning of tafamidis therapy, and the mean follow-up time at first ophthalmologic evaluation was 4.8±3.3 years. At the end of the study (February, 2016), amyloid deposits on anterior lens capsule were present in 27 right eyes and in 22 left eyes, amyloid deposits on pupillary border was present in 21 and 18 right and left eyes and scalloped pupil in 8 right and 11 left eyes. Amyloid vitreous opacities were present in 36 right and left eyes.

Mean onset age was significantly higher in female gender (46±12 vs. 36±13 years, P=0.011). No significant changes were found in gender in relation to mean evolution time of the disease at the first time of evolution.

Female gender was significantly associated with higher risk (higher hazard ratio) of amyloid deposits on lens anterior capsule, amyloid deposits on pupillary border, and vitreous opacities, independently of the onset age and the evolution time of the disease. The higher mean onset age was also statistically significant associated with a higher risk of scalloped pupil, amyloid deposits on anterior lens capsule, amyloid deposits on pupillary border, and vitreous opacities. No significant association was found between years of evolution of the disease and the ophthalmologic events. The interaction between gender and onset age was also tested but it was removed from all model due to non-significance.

Conclusions

Female gender and higher onset age are independent predictors of ophthalmological changes, namely amyloid deposits on lens anterior capsule, amyloid deposits on pupillary border, scalloped pupil, and vitreous opacities.
Early cardiac findings in Val30Met transthyretin-related hereditary amyloidosis in a Brazilian population

AC Berensztejn, RC Pedrosa, MC Queiroz, M Waddington-Cruz

Federal University of Rio de Janeiro, National Amyloidosis Referral Center, CEPARM, Rio de Janeiro, Brazil. amandacard@gmail.com

INTRODUCTION: Transthyretin-related hereditary amyloidosis is a rare, heterogeneous disease, primarily characterized by neurologic and cardiac involvement. There are more than 100 different mutations described, with Val30Met the prevalent mutation in Brazil, an endemic region due to its Portuguese colonization. These patients predominantly present with a neurological phenotype, with cardiac manifestations occurring later during the course of the disease. We aimed to describe cardiac findings in this population of patients under 45 years of age with the Val30Met mutation presenting with initial symptoms of progressive peripheral sensory-motor and autonomic neuropathy.

MATERIAL & METHODS: Records of 39 patients under 45 years of age with the Val30Met mutation were collected, including demographic (including inheritance profile), clinical characteristics, echocardiographic, electrocardiographic, and 24h-Holter monitoring findings. The outcome measures included demographics, clinical characteristics (presenting symptomatology, NYHA classification) and cardiac echocardiographic, electrocardiographic, and 24h-Holter monitoring findings.

RESULTS: 39 subjects (59% male) were included in the analysis with a median age at the evaluation time of 36.6 years. Of the studied cases, 56.4% had paternal inheritance. Echocardiographic abnormalities were found in 20% of the cases, some presenting with increased ventricular wall thickness and granular sparkling appearance of the myocardium. Electrocardiographic abnormalities were present in 41%, including conduction blocks and low voltage.

DISCUSSION & CONCLUSIONS: The Brazilian population of subjects with TTR amyloidosis is mainly characterized by Val30Met mutation and a neurological phenotype. In spite of that, we have observed that early cardiac amyloidosis may remain undiagnosed with the use of 12 mm as the cutoff for LV mural thickness. Cardiac amyloidosis can be an early finding in this population under 45 years of age with a neurological phenotype.

Fig. 1: Echocardiographic image of a 31-year-old female subject with Val30Met hereditary amyloidosis, demonstrating increased left ventricular wall thickness and granular sparkling.
Gender differences and age-at-onset distributions in Familial Amyloid Polyneuropathy (FAP ATTRV30M): a two-story tale

A Sousa1,2, C Lemos1,2, M Alves-Ferreira1,2, D Santos1,2, J Sequeiros1,2, D Mendonça2,3, T Coelho4

1 UnIGENe, Institute for Molecular and Cell Biology (IBMC) and Instituto de Investigação e Inovação em Saúde (i3S), Univ. Porto, Porto, Portugal
2 Instituto Ciências Biomédicas Abel Salazar (ICBAS), Univ. Porto, Porto, Portugal
3 ISPUP, Instituto de Saúde Pública, Univ. Porto, Porto, Portugal
4 Unidade Corino de Andrade (UCA), Centro Hospitalar do Porto (CHP), Porto, Portugal.

INTRODUCTION: Familial Amyloid Polyneuropathy (FAP) ATTRV30M is an autosomal dominant systemic amyloidosis due to a point mutation in the transthyretin (TTR) gene. First described in Portugal by Andrade (1952) as a disease of young adults (age-at-onset - AO <40yrs), Portuguese patients have been characterized by early onset (35.4yrs.), unlike patients from Sweden (56.7yrs) and Balearic Islands (45.7yrs), who bear the same mutation. However, late-onset patients (AO ≥50) and aged asymptomatic carriers have been increasingly ascertained. AO in Portugal varies from 19 to 82yrs and there is evidence of asymptomatic carriers aged over ninety whose children are affected at various ages.

In Portuguese series women have later onset than men, although the same was not found either in Swedish or Balearic samples. This raises interesting questions concerning gender and AO distribution(s).

MATERIAL & METHODS: From the data registry at Unidade Corino de Andrade, we retrieved data on 2713 patients (1461 men) and 604 (212 men) proven asymptomatic carriers regularly followed up by the same group of neurologists. Data included gender, AO (for patients) and age-at-last observation (for asymptomatic carriers). We used t-test and Mann-Whitney test to compare AO and age-at-observation between men and women. Furthermore, we used survival analysis methods to estimate AO, including in the sample, as censored data, age-at-last-observation of asymptomatic carriers. Survival curves were estimated by Kaplan-Meyer methods and compared by gender using the log-rank test. Statistical analysis was performed using SPSS v.23 software.

RESULTS: Conventional t-test for independent samples showed significant differences in mean AO between men (33.4) and women (37.6) patients (p<0.001), whereas in the asymptomatic group no significant gender differences were found for age-at-last observation (38.0 vs. 38.6). Also, we found that while in the early-onset group women had later onset than men (p<0.001), in the late-onset group, men had a higher onset than women (p<0.005). Identical results were found when using Mann-Whitney test.

We then used survival analysis including the asymptomatic carriers as censored data: log-rank tests showed overall different gender distributions and also when considering individuals with onset/age<40yrs. However, for cases with onset/age≥40yrs, no significant differences were found.

DISCUSSION & CONCLUSIONS: The use of Survival analysis to estimate AO of genetic diseases has proven useful, since it incorporates individuals who are unaffected at a given age. In our case, the large sample of FAP patients and of asymptomatic carriers on follow-up allows a correction to AO: probably due to a pool of older asymptomatic women, gender differences are no longer apparent in the group with onset/age ≥40, whereas conventional t-tests showed gender differences in opposite directions in the group with AO<40 and in the group with AO≥40.

Next step should be to further explore survival analysis to disentangle if we are in presence of more than one underlying distribution for each gender.
Intergenerational variability of age-at-onset of Familial Amyloid Polyneuropathy (FAP ATTRV30M): what is hidden in late-onset?

C. Lemos1,2; T. Coelho3; M. Alves-Ferreira1,2; D. Santos1,2; J. Sequeiros1,2; A. Sousa1,2

1 UnIGENe, Institute for Molecular and Cell Biology (IBMC) and Instituto de Investigação e Inovação em Saúde (i3S), Univ. Porto. 2 Instituto Ciências Biomédicas Abel Salazar (ICBAS), Univ. Porto. 3 Unidade Corino de Andrade (UCA), Centro Hospitalar do Porto (CHP), Porto, Portugal.

clclemos@ibmc.up.pt

INTRODUCTION: Familial Amyloid Polyneuropathy (FAP) is an autosomal dominant systemic amyloidosis, due to a point mutation in the transthyretin (TTR) gene, being V30M the commonest. In a previous study in Portuguese patients, with affected parent-offspring pairs, we confirmed anticipation - a much earlier age-at-onset (AO) in offspring than their affected parent - as a true biological phenomenon1. Furthermore, we found that both parents and offspring’s gender were highly significant factors for anticipation. We also found familial aggregation of aged-asymptomatic carriers and late-onset cases. Our aim now is to deepen our previous analysis and to further explore families with late-onset cases.

MATERIAL & METHODS: From the most recent update on the data registry of patients at Unidade Corino de Andrade, Porto, we started from a cohort of late-onset cases (≥50) and we analysed their affected offspring and further their own offspring (3rd generation). Categorical variables were assessed using Chi-square test and Mann-Whitney test was used to compare age-at-observation between groups. Statistical analysis was performed using SPSS v.23 software.

RESULTS: Of a total of 178 offspring (96 males and 82 females), we found 105 cases (59%) with an early AO (<40), of which 59% were males and 41% were females. Moreover, 65% inherited the disease from the mother while 35% inherited from the father and this difference is statistically significant. On the contrary, 73 presented an AO ≥40 (47% males, 53% females) and there are no differences regarding parent-of-origin. Another interesting feature is that most of the siblings in these sibships presented an early AO, with only some exceptions, showing a high concordance in the onset of the disease. In the 3rd generation, we found that only two individuals (2.9%) presented an AO ≥ 40, while 30 were asymptomatic (44.1%). The remaining 36 (52.9%) presented an early AO. From the asymptomatic group, we found that they have a higher age-at-observation when the transmitting parent had an AO ≥40. We did not find any offspring in the second and third generation that had a later onset than the 1st generation.

DISCUSSION & CONCLUSIONS: Our starting point was a cohort of late-onset cases and our aim was to characterize AO in the subsequent generations since this is a unique opportunity to study AO in three generations with FAP ATTRV30M. What we found is that after a decrease in AO is observed, the reverse is not found in any of the families observed. As described in our previous study1, the early-AO offspring inherits the disease from the mother while 35% inherited from the father and this difference is statistically significant. On the contrary, 73 presented an AO ≥40 (47% males, 53% females) and there are no differences regarding parent-of-origin. Another interesting feature is that most of the siblings in these sibships presented an early AO, with only some exceptions, showing a high concordance in the onset of the disease. In the 3rd generation, we found that only two individuals (2.9%) presented an AO ≥ 40, while 30 were asymptomatic (44.1%). The remaining 36 (52.9%) presented an early AO. From the asymptomatic group, we found that they have a higher age-at-observation when the transmitting parent present a late AO. Due to different clinical aspects of FAP in late-onset patients and asymptomatic carriers and the sudden decrease in AO in just one generation it is crucial to explore mechanisms that can be related with aging and genetic and epigenetic factors involved in AO variability.

Insights from novel understanding of pathogenesis in transthyretin amyloidosis

PP Mangione1,2, G Verona1,2, R Porcari2, G Faravelli1, S Giorgetti1, GW Taylor2, JD Gillmore2, PN Hawkins2, MB Pepys2, V Bellotti1,2

1 Department of Molecular Medicine, University of Pavia, Pavia, Italy.
2 Centre for Amyloidosis and Acute Phase Proteins, University College London, London, UK
p.mangione@unipv.it

INTRODUCTION: Cardiac amyloidosis in the elderly, caused by wild type transthyretin (TTR), is increasingly recognized as an important clinical problem but the molecular mechanisms of TTR fibrillogenesis in vivo remain unclear. Dissociation of the native TTR tetramer is believed to be a prerequisite for formation of TTR amyloid fibrils but the C-terminal, residue 49-127, TTR fragment is notably present in most ex vivo TTR amyloid deposits [1] and is associated with both cardiac amyloid deposition and poor clinical prognosis [2]. Small ligands bound by the thyroxine binding sites of TTR, thereby stabilizing the native tetramer, are currently used to treat ATTR amyloidosis. Despite effectively inhibiting TTR dissociation in vitro, their in vivo efficacy is limited. We have investigated this unexplained discrepancy, focusing on the robustly reproducible formation of abundant, authentic amyloid fibrils from TTR under physiologically relevant conditions.

MATERIALS & METHODS: The role of the residue 49-127 TTR fragment and of shear stress in the formation of TTR amyloid fibrils under physiological conditions in vitro was comprehensively investigated using classical biochemical techniques, including limited proteolysis, electrophoresis, size exclusion chromatography and thioflavin T fluorescence. The authentic amyloid fibrils that were generated were characterized by histochemical and microscopic analyses.

RESULTS: We have identified a previously unrecognised mechano-enzymatic mechanism responsible for TTR amyloid fibrillogenesis, in which the residue 49-127 TTR fragment, generated under physiological conditions, is released from the tetramer and then promotes rapid fibril formation [3,4]. Existing TTR stabilizers, such as tafamidis and diflunisal, inhibit proteolysis-mediated TTR fibrillogenesis but with different potency for each TTR variant. The best inhibitors are those lacking negative cooperativity as well as palindromic ligands which are pseudo-irreversibly bound by TTR.

DISCUSSION & CONCLUSIONS: Our more precise elucidation of the actual mechanism by which TTR forms amyloid fibrils under physiological conditions should open the way to design of potentially more effective drugs and will also enable better interpretation of clinical and biochemical endpoints in current and future clinical trials.

REFERENCES:
Long-term effect of Tafamidis in Transthyretin Familial Amyloid Polyneuropathy: A study in Val30Met and non-Val30Met patients

V Planté-Bordeneuve 1,2, F Gorram1,2, H Salhi1,2, T Nordin1,4,5, S Ayache1,4,5, P Le Corvoisier1,3, D Azoulay1,6, C Feray1,6, T Damy1,7, J-P Lefaucheur1,4,5

1 Amyloid Network, Henri Mondor University Hospital, AP-HP, Créteil, France. 2 Department of Neurology, Henri Mondor University Hospital, AP-HP, Créteil, France. 3 Clinical Investigation Center 1430, IMRB, Henri Mondor University Hospital, Inserm, Créteil, France. 4 Clinical Neurophysiology Unit, Department of Physiology, Henri Mondor University Hospital, AP-HP, Créteil, France. 5 EA 4391, Faculty of Medicine, Paris-Est Créteil University, Créteil, France. 6 Liver Transplant Unit, Department of General Surgery, Henri Mondor University Hospital, AP-HP, Créteil, France. 7 Heart Failure Unit, Department of Cardiology, Henri Mondor University Hospital, AP-HP, Créteil, France.

Violaine.plante@aphp.fr

Background: Tafamidis is an approved treatment in Europe and Japan for patients with familial amyloid polyneuropathy (FAP) related to a mutation in the transthyretin (TTR) gene. This drug is able to stabilize the structure of the TTR variant. Tafamidis is indicated in TTR-FAP at an early stage with clinical effect mainly studied in Val30Met patients. Conversely, little is known about the therapeutic value of tafamidis in patients at a more advanced stage or with non-Val-30Met mutation.

Aim: To report experience of the long-term use of tafamidis (average duration of treatment 36 months)

Methods: We evaluated prospectively patients diagnosed TTR-FAP and treated by tafamidis 20 mg/day in our center from 2010 to 2014. Longitudinal data were collected at 6-12M, 18-24M and 30-36 months after treatment initiation. Investigated parameters were gender, age at disease onset and treatment initiation, disease duration before treatment, type of TTR mutation, body mass index (BMI), the Neuropathy Impairment Score (NIS), the modified Polyneuropathy Disability Score (mPND), the Karnofsky performance status (KPS), and a large battery of neurophysiological tests specific for large (LFNS) or small nerve fibre assessment (SFNS). Correlations between ANIS at each periods and baseline data were analyzed concerning qualitative (i.e. Val30Met vs non Val30Met, gender) and quantitative variables (i.e. age at disease onset, disease duration before treatment, BMI, NIS, mPND, KPS, LFNS, and SFNS).

Results: There were 43 patients of various severity and duration due to different TTR mutations (47% of Val30Met variant and 53% of non-Val30Met variants). Age at disease onset ranged from 31.0 to 79.0 years (mean ± sem: 59.2 ± 2.0). The main results were as follows (i) patients continued to clinically deteriorate under tafamidis, as soon as the first year of treatment and at the same rate over the next two years; (ii) clinical deterioration mainly correlated to older age at disease onset or treatment initiation and to worse clinical status of TTR-FAP at baseline; (iii) the response to treatment was mainly associated with a younger age at treatment initiation and a higher BMI at baseline; (iv) about 30-40% of the patients who still received tafamidis at the end of the 36 months follow-up had preserved walking capacity (v) on neurophysiological ground, there was a stability or slight improvement within the first two years of treatment, but a significant deterioration during the third year.

Conclusion: This is the first report of the long-term use of tafamidis in patients with a large pheno-genotypic spectrum of TTR-FAP. Our study suggests that a better control of TTR-FAP progression could be achieved if the treatment is administered to younger patients, with a little advanced TTR-FAP and a higher BMI at baseline. Such clinical characteristics appear to be better predictor of treatment efficacy than the type of TTR variant. A better selection of patients could improve the control of TTR-FAP progression by tafamidis.
Unraveling penetrance estimates in the main variants of Transthyretin Familial Amyloid Polyneuropathy by use of a non-parametric approach

F Gorram1,2,3, F Alarcon4, H Perdry5, B Hébrard4, T Damy2,3,7, P Fanen3,6, B Funalot3,6,8, G Nuel9 and V Planté-Bordeneuve1,2,3

1 Department of Neurology, Henri Mondor University Hospital, AP-HP, Créteil, France. 2 Amyloid research Institute, Amyloid Network, Créteil, France. 3 Laboratory MAP5 UMR CNRS 8145 Paris Descartes University, France. 4 University Paris-Sud, UMR-S 669 - Inserm, U669, Villejuif, France. 5 Department of Cardiology, Henri Mondor University Hospital, AP-HP, Créteil, France. 6 Department of genetics, Henri Mondor University Hospital, AP-HP, Créteil, France. 7 Department of Cardiology, Henri Mondor University Hospital, AP-HP, Créteil, France. 8 Institute of Mathematics, National Center for French Research, Laboratory of Probability, University Pierre et Marie Curie, Sorbonne University, France.

Violaine.plante@aphp.fr

Background: Significant variability of phenotype and age of onset are well known in transthyretin familial amyloid neuropathy (TTR-FAP) to be associated to a wide spectrum of pathogenic TTR mutations, among which Val30Met is the most frequent. Recently, new therapeutic options for TTR-FAP became available but should be administered from the onset of symptoms. In this context, the knowledge of the risk of being symptomatic for mutation carriers (penetrance of the disease) is essential to ensure a more accurate follow-up of carriers and for patient management.

The present study aims to refine estimates of penetrance in the main pathogenic TTR variants encountered in our TTR-FAP population using a newly developed non parametric approach.

Methods: A systematic genealogical enquiry was carried out in each family assessed in our center. Relevant data were collected with special attention to the genotype, phenotype, age at onset for affected individual and age at last news for asymptomatic carriers and relatives. Portuguese families were not included here.

Previously, we estimated penetrance as function of age, with the parametric survival method PEL (Proband’s Exclusion Likelihood), in which the age at onset is modeled by a Weibull distribution (WD). However, PEL can fail to fit properly the survival function when values of penetrance are far from the WD. To avoid these biases we have developed a non-parametric method that uses a Kaplan-Meier estimator allowing to fit without bias any penetrance shape. This method also allows to test covariates such as gender, or mutations and perform comparisons using a log rank test.

Results: We obtained genealogical data from 71 unrelated kindreds, including Val30Met (35 families, 90 patients), Ser77Tyr (15 families, 47 patients), Ile107Val (12 families, 21 patients), Ser77Phe (9 families, 30 patients) with information on 1654 subjects (188 affected /115 asymptomatic carriers).

Mean age at onset (SD) was 54.6 years (14.9) for Val30Met, 55.6 years (9.1) for Ser77Tyr, 62.3 years (6.8) for Ile107Val, and 58.7 years (6.4) for Ser77Phe.

Penetrance estimates were significantly different between the 4 TTR variants tested (p= 0.003) and remained incomplete in older patients. By the age of 80 years, a wide range of penetrance was observed, from 46.9% [16.6; 66.2] in Ile107Val up to 71.3% [51.1; 83.1] for Ser77Tyr. The disease risk was virtually null until the age of 50 years for all variants except for the Val30Met group where it increases progressively from 3.1% [0.3 ; 5.7] at 30 years to 16.5% [9.8 ; 22.7] at 50 and 64.4% [49.4 ; 74.9] at 80 years old. In contrast, the risk increased abruptly after 50 years old in Ser77Phe and Ser77Tyr carriers, from 7% [0.8; 15.8] and 12.8% [3.3; 21.4] respectively to 65.8% [37.1; 81.4] and 71.3% [51.1; 83.1] at 70 years old. Risk estimate was found the lowest in Ile107Val families where it increases slightly from 50 years to reach 10.5% [0.5; 21.1] at 60 and 37.5% [14.2 ; 54.4] at 70 years old.

Conclusion: This study showed significant differences of penetrance profiles in TTR-FAP with various pathogenic mutations of TTR. Our results should be replicated and implemented on larger samples of families to refine the management of carriers and better understand the disease expression.
Peripheral polyneuropathy in wild type transthyretin cardiac amyloidosis: initial observations in a new prospective study

P. James B. Dyck, M.D.1, Martha Grogan, M.D.2, Merrill D. Benson, M.D.3, Morie A. Gertz, M.D.4, Christopher J. Klein, M.D.1, William J. Litchy, M.D.1, Michelle L. Mauermann, M.D.1, JaNean K. Engelstad, H.T.1, Jenny L. Davies1 and Peter J. Dyck, M.D.1

Department of Neurology, Mayo Clinic, Rochester, MN 1; Department of Cardiovascular Diseases, Mayo Clinic, Rochester, MN2; Department of Pathology and Laboratory Medicine, Indiana University, Indianapolis, IN3; Department of Hematology, Mayo Clinic Rochester, MN4
dyck.pjames@mayo.edu

INTRODUCTION: Both mutant and wild transthyretin (TTR) are common constituents of tissue amyloid deposits in familial amyloid polyneuropathy (FAP) caused by more than 100 mutations of TTR. We have observed signs and symptoms of polyneuropathy in patients with wild type transthyretin cardiac amyloidosis (ATTRwt). The objective of this study is to determine the frequency of polyneuropathy in ATTRwt,

MATERIAL & METHODS: Patients with ATTRwt were referred for a neurological evaluation. Neuropathic signs were assessed by Neuropathy Impairment Score (NIS). Symptoms were assessed by history and Neuropathy Symptom and Change (NSC). Nerve conduction studies (NCS) were performed on selected nerves. Touch pressure and heat as pain were assessed by Smart Somatotopic Quantitative Sensation Testing (ST QSTing). Three mm skin punch biopsies to estimate ENFDs and deposits of amyloid were obtained from dorsal foot, lateral leg or mid thigh. QSWEAT and HRDB were obtained in a subset of patients.

RESULTS: Seven patients with cardiomyopathy from ATTRwt were evaluated. Five patients had mild symptoms and four had mild findings of a sensory polyneuropathy. One patient had abnormal summated NCS. Three patients had quantitative sensation test abnormalities. Decreased epidermal nerve fiber densities were found in two patients. Amyloid deposition was found in five of seven patients, and was the distal site in 3 and in the proximal site in 2 patients. Figure 1 shows amyloid deposition in one patient (sections A – C) and reduced ENFD in another (section D).

DISCUSSION: Symptoms and findings of polyneuropathy were observed in over half (4 of 7) of the studied patients with ATTRwt. Tissue deposits of TTRwt in skin were observed in 5 of 7 cases. Multiple skin biopsy sites may be necessary to detect amyloid deposition. We plan to prospectively evaluate 25 – 50 additional patients with ATTRwt to better define this association.

CONCLUSIONS: Our preliminary results demonstrate that polyneuropathy occurs in ATTRwt patients. A larger cohort of patients will be studied to determine the frequency and severity of polyneuropathy in patients with cardiac amyloid due to ATTRwt.

Fig. 1. Transverse paraffin sections of skin punch biopsies (A-C): A) H&E, low power, B) Congo red showing large deposit of amyloid (arrow), C) apple-green birefringence under polarizing filters (arrow) and D) skin punch biopsy section reacted with PGP 9.5 showing reduced density of epidermal nerve fibers.
Doxycycline treatment of amyloidosis: a phase II study

JL Berk1,3, FL Ruberg1,3, S Pawar3, CA Brueckner1, JF Wiesman1,4, L Stern1,3, A Havasi1,3, JE Ward1, V Sanchorawala1,3

1Amyloidosis Center; 2Section of Cardiovascular Medicine, 3Department of Medicine; 4Department of Neurology, Boston Medical Center, Boston USA.

jberk@bu.edu

INTRODUCTION: The tetracycline antibiotic doxycycline disrupts A beta amyloid fibrils (AB) in Alzheimer’s disease, transthyretin (ATTR) amyloid fibrils in familial amyloidotic polyneuropathy, and immunoglobulin light chain (AL) amyloid fibrils in transgenic mouse models of disease.1,2 Untreated, amyloid deposits impair organ function and lead to death. We conducted a single arm open label phase II study to determine whether doxycycline can reduce amyloid deposits in localized disease and improve organ function in patients with systemic amyloidosis.

MATERIAL & METHODS: Patients with amyloid-related measurable end organ disease not requiring active treatments for precursor protein production were eligible for study participation. Organ involvement was based on tissue sampling or consensus criteria.3 Amyloid type was determined by immunohistochemical staining or mass spectrometry. All enrolled received open label doxycycline 100 mg taken twice daily by mouth. Measures of the principally affected organ were obtained at baseline, 6 and 12 months along with safety/toxicity laboratories (complete blood count, liver enzymes, serum electrolytes). Assessments included 24 h urine collections for creatinine clearance (CrCl) and protein excretion (renal amyloid), liver enzymes and abdominal computerized tomography (CT) (liver amyloid), B-type natriuretic protein (BNP), troponin I (Tnl), metabolic stress testing, and echocardiographic parameters (amyloid cardiomyopathy), spirometry, stair climbing, arterial blood gases, airway CT imaging (tracheobronchial amyloidosis), confrontational neurologic scoring (facial neuropathy). The percent change in principal organ measures from baseline was determined at 6 and 12 months. A 50% improvement in principal organ functional deficit constituted drug effect. Statistical significance was set at a two-sided alpha=0.05.

RESULTS: We enrolled 25 patients with amyloidosis aged 65 years (median, range 36-82) including 34% women, 12 cardiomyopathy (3 AL, 3 ATTRm, 6 ATTRwt), 10 nephrotic range proteinuria (6 AL, 2 ApoAI/II, 1 Gelsolin, 1 Lysozyme), 1 subglottic airway disease (AL), 1 facial neuropathy (Gelsolin), 1 hepatopathy (AL). Of the 107 adverse events, 17% were GI and 20% were dermatologic. Fourteen patients completed 12 months treatment; 11 discontinued doxycycline, 3 due to drug-related events. Severe AEs (SAE) occurred in 12 patients (48%), 3 due to doxycycline. The renal amyloid subgroup exhibited no change in proteinuria over 12 months (median change -0.22%, range -60 to 9.49%) with a small decline in CrCl (median -9.97%, range -2.30 to -58.63%). In the cardiac amyloid group, BNP and Tnl rose (median 72.25%, range -7.99 to 144.5%, and median 28.57%, range -10 to 82.76%, respectively), echo (IVSd, LVEF, lateral e’), and metabolic stress test parameters (maximal oxygen uptake, VE/VCO2 slope, METS) remained unchanged. Plasma cell dyscrasia markers for AL amyloidosis were stable throughout the 12 month doxycycline treatment.

CONCLUSIONS: Doxycycline treatment of patients with measurable amyloid end organ disease induced no significant percentage improvement in organ function by multiple testing over a period up to 12 months. Sun hypersensitivity and GI complaints limited doxycycline administration in over 30% of our cohort. A placebo controlled experience would be needed to determine whether end organ stability signals drug benefit.

Decreased physical quality of life increases the risk of death in patients with familial transthyretin amyloidosis

V Lattanzi1, HJ Cabral2, LH Connors1,3, FL Ruberg1,4, Wiesman JF1, JL Berk1

1Amyloidosis Center, Boston University School of Medicine; 2Department of Biostatistics, Boston University School of Public Health; 3Department of Pathology and Laboratory Medicine, Boston University School of Medicine; 4Section of Cardiovascular Medicine, Boston Medical Center, Boston, USA.

INTRODUCTION: Point mutations in the transthyretin (TTR) gene destabilize circulating tetrameric TTR, inducing protein misaggregation, amyloid formation, and the clinical manifestations of familial transthyretin amyloidosis (ATTRm). Untreated, death occurs 7-15 years after presentation. In AL and familial ATTR amyloidosis, quality of life (QOL) using the 36 question short form health survey (SF-36) directly relates to disease course and treatment outcome. However there are few data correlating QOL data with survival. We determined the QOL of ATTRm patients at presentation and its association with survival.

MATERIAL & METHODS: SF-36 QOL questionnaires were systematically collected between 1985 and 2015 at presentation and follow up visits to the Amyloidosis Center at Boston University from patients with ATTRm disease. All patients had tissue amyloid deposits by Congo red staining, and TTR gene sequencing to establish the specific mutation. The SF-36 instrument includes 8 scales of health status -- physical functioning (PF), role limiting physical problems (RP), bodily pain (BP), social functioning (SF), mental health (MH), role limiting emotional problems (RE), vitality (VT), and general health (GH) – expressed as physical (PCS) and mental (MCS) component summaries. PCS and MCS scores were standardized to the adult US population by age and gender. Using hazard ratios in Cox survival models, we examined the association of PCS and MCS at presentation and follow up with survival and cardiac biomarkers, adjusting for age at presentation and comorbidities including diabetes mellitus, hypertension, hyperlipidemia, and gender. Statistical significance was set at a two-sided alpha=0.05.

RESULTS: We analyzed presenting SF-36 data from 331 patients with biopsy and genomics proven ATTRm. The cohort was 67% male, 82% Caucasian, and 14% African American with an age of 57.7 ± 13.9 years (mean ± SD) at presentation. The initial mean MCS and PCS scores were 45.2 ± 11.7 and 37.2 ± 13.3, respectively, 0.5 and 1.5 SD below age and gender matched scores from a standard US population. During follow up 120 deaths occurred. In a Cox survival analysis adjusted for age at presentation and comorbidities, patients with PCS scores < 35 had a significantly higher risk of death during follow up than those with scores > 35(hazard ratio (HR) 2.76 (p= <0.0001). In contrast, an MCS score < 35 did not correlate with increased risk of death during follow up (HR1.38, p=0.13). By Spearman rank analysis, brain natriuretic peptide (BNP) and troponin I most strongly associated with PCS (r= -0.50, r=-0.41, respectively) and less with MCS (r= -0.16 and r= -0.24, respectively).

CONCLUSIONS: At presentation, ATTRm disease significantly decreases a patient’s physical quality of life with lesser decrements of mental health scores. Patients with physical QOL scores more than one and one half standard deviations below age and gender standardized US population had a significantly greater risk of death during follow up than those with higher QOL scores. Similar magnitude deficits of mental QOL did not associate with increased risk of death. We conclude that ATTRm preferentially decreases patients’ physical QOL and survival.


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Patients with wild-type transthyretin amyloidosis and decreased quality of life are at increased risk of death

V Lattanzi, HJ Cabral, LH Connors, FL Ruberg, JL Berk

Amyloidosis Center, Boston University School of Medicine, Boston, USA. Department of Biostatistics, Boston University School of Public Health, Boston, USA. Department of Pathology and Laboratory Medicine, Boston University School of Medicine, Boston, USA. Section of Cardiovascular Medicine, Boston Medical Center, Boston, USA.

INTRODUCTION: Wild-type transthyretin amyloidosis (ATTRwt) arises from the misaggregation and tissue deposition of a normal hepatic transport protein. Amyloid deposition typically in the hearts of older men induces organ dysfunction, functional disability, and death. In AL and familial ATTR amyloidosis, quality of life (QOL) using the 36 question short form health survey (SF-36) correlates with disease course and treatment outcome. Few QOL data exist in ATTRwt disease. We determined the QOL of ATTRwt patients at presentation and its association with survival.

MATERIAL & METHODS: We reviewed SF-36 QOL questionnaires systematically collected between 1994 and 2015 at presentation and follow up visits to the Amyloidosis Center at Boston University from patients with ATTRwt disease. All patients underwent amyloid tissue typing and TTR gene sequencing to confirm the diagnosis. The SF-36 instrument includes 8 scales of health status -- physical functioning (PF), role limiting physical problems (RP), bodily pain (BP), social functioning (SF), mental health (MH), role limiting emotional problems (RE), vitality (VT), and general health (GH) -- expressed as physical (PCS) and mental (MCS) component summaries. PCS and MCS scores were standardized to the adult US population with respect to age and gender. Using hazard ratios in Cox survival models, we examined the association of PCS and MCS at presentation and follow up with survival, modified body mass index, and cardiac disease, adjusting for age at presentation, and comorbidities including diabetes mellitus, hypertension, hyperlipidemia, and prior coronary artery disease or myocardial infarction. Statistical significance was set at a two-sided alpha=0.05.

RESULTS: We analyzed SF-36 data from 133 white males with proteomic and genomic proven ATTRwt amyloidosis aged 74.7 ± 6.1 years (mean ± SD) with a BMI (28.5 ± 4.0), and New York Heart Association (NYHA) of class I (19%), II (43%), and III-IV (37%). The mean MCS and PCS scores were 45.7 ± 12.3 and 36.7 ± 10.8, respectively, and inversely related to NYHA class. From presentation to 2016 censor, 54% of the cohort died. In a Cox survival analysis adjusted for age at presentation and comorbidities, patients with PCS or MCS scores < 35 had a significantly higher risk of death during follow up than those with scores > 35 (hazard ratio (HR) 2.45 (p=0.002) for PCS; HR 3.38 (p<0.0001) for MCS).

CONCLUSIONS: At presentation to an amyloid center of excellence, ATTRwt disease significantly decreases a patient’s physical and mental quality of life. Patients with mental or physical QOL scores more than one and one half standard deviations below the age and gender standardized US population had a significantly greater risk of death during follow up than those with higher QOL scores. We conclude that ATTRwt adversely affects patient’s QOL and survival.

REFERENCES:


Supported by National Institutes of Health RO1AG031804, and the Young Family Amyloid Research Fund
The role of a change in ECG voltage in the diagnosis and prognosis of patients with cardiac amyloidosis

BW Sperry1, I Bagh2, MN Vranian1, M Hanna1
1Department of Cardiovascular Medicine and 2Department of Internal Medicine, Cleveland Clinic Foundation, Cleveland Ohio
sperryb@ccf.org

INTRODUCTION: Low voltage on ECG may be caused by various medical conditions. In cardiac amyloidosis, low voltage in the limb leads is found in approximately 50% with AL and only 20-25% of ATTR, whether senile or familial. Despite not meeting low voltage criteria on ECG, a reduction of voltage over time may signify amyloid cardiomyopathy.

MATERIALS & METHODS: The electrocardiograms of consecutive patients at the time of a diagnosis of cardiac amyloidosis at our institution were analyzed. Patients with ventricular paced rhythms were excluded. An additional ECG at least one year prior to the diagnosis was also analyzed. The sum of voltage in all limb leads and the Sokolow voltage (S wave in V1 plus R wave in V5 or V6) index were calculated. Low limb voltage was defined as ≤ 5 mm in all limb leads and low precordial voltage as ≤ 10 mm in all precordial leads. Change in voltage was defined change in voltage divided by years between studies. A Cox proportional hazards model was used to assess the association between decreasing ECG voltage and 3 year mortality.

RESULTS: A total of 338 patients (192 AL, 146 ATTR, age 68.7 +/- 12.1 years, obese 57%, HTN 54%) were identified. At the time of diagnosis, 167 patients (49.4%) met either low limb or low precordial voltage criteria. The limb lead voltage decreased in 63 of 73 patients (86.3%) and the Sokolow voltage decreased in 62 of 73 patients (84.9%). The mean overall decrease in limb lead voltage was 10.6 +/- 12.6 mm overall or 3.3 +/- 4.6 mm per year after normalizing for time between exams. The Sokolow voltage decreased 6.5 +/- 7.1 mm overall or 1.8 +/- 2.3 mm per year. On multivariable analysis, the annualized decrease in limb voltage was significantly associated with mortality after adjusting for limb and Sokolow voltage at the time of diagnosis, age, hypertension, GFR, BSA and AL subtype (Table 1).

DISCUSSION & CONCLUSION: A decrease in voltage on serial ECG’s is more prevalent than low voltage in cardiac amyloidosis. A higher annual decrease in ECG limb voltage was associated with mortality after multivariable adjustment. The evaluation of serial ECG’s for changes in voltage is important to recognize in the diagnosis of cardiac amyloidosis and may have prognostic value.

Table 1: Cox proportional hazards model depicting association between change in limb voltage and mortality

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio</th>
<th>CI (95%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limb voltage</td>
<td>0.987</td>
<td>0.957-1.018</td>
<td>0.398</td>
</tr>
<tr>
<td>Sokolow voltage</td>
<td>0.980</td>
<td>0.925-1.039</td>
<td>0.500</td>
</tr>
<tr>
<td>Δ Sokolow/yr</td>
<td>1.038</td>
<td>0.854-1.260</td>
<td>0.709</td>
</tr>
<tr>
<td>Δ Limb/yr</td>
<td>0.919</td>
<td>0.849-0.994</td>
<td>0.035</td>
</tr>
<tr>
<td>AL</td>
<td>5.869</td>
<td>2.432-14.161</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>1.058</td>
<td>1.016-1.103</td>
<td>0.007</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.634</td>
<td>0.785-3.400</td>
<td>0.189</td>
</tr>
<tr>
<td>eGFR</td>
<td>0.985</td>
<td>0.971-0.999</td>
<td>0.031</td>
</tr>
<tr>
<td>BSA</td>
<td>0.313</td>
<td>0.062-1.570</td>
<td>0.158</td>
</tr>
</tbody>
</table>
Assessment of the utility of genetic testing in amyloid subtyping

EF Brown¹, NM Johnson¹², J Almansa¹, K Sharma¹, DP Judge¹

¹ Department of Cardiology, Johns Hopkins University, Baltimore, Maryland. ² Invitae Corporation, San Francisco, California.

INTRODUCTION: Incorrect subtyping of amyloidosis can lead to inappropriate treatment and inadequate counselling for family members who are at risk for this condition. Traditionally, amyloid subtyping has been performed on biopsy-positive tissues using immunohistochemistry (IHC). However, IHC has been shown to have limited diagnostic yield, with one study showing 24% of cases could not be subtyped by IHC alone [1]. Laser microdissection and mass spectrometry to determine the subtype has been shown to increase detection by 18% [1]. The use of liquid chromatography tandem mass spectrometry (LC MS/MS) and proteomics has allowed laboratories to further characterize the subtype by identifying mutant peptides. Proteomic analysis has been shown to have a sensitivity of 92-100% and as a result, has been suggested to replace genetic testing for amyloid subtyping [2,3].

MATERIAL & METHODS: A retrospective review identified 144 patients diagnosed with biopsy proven transthyretin amyloidosis in the cardiomyopathy clinic at Johns Hopkins from 2003 to 2016. Seventy three patients were diagnosed with hereditary transthyretin amyloidosis, and 71 patients were diagnosed with wild-type transthyretin amyloidosis. Of the 73 patients with hereditary disease, 48 patients had both genetic testing and LC MS/MS. The LC MS/MS was performed by the Mayo Clinic, and the genetic testing was sent to CLIA certified laboratories.

RESULTS: LC MS/MS and proteomics correctly identified the mutant peptide and heterozygosity in 41/48 (86%) cases. One patient was a compound heterozygote for the mutations Val122Ile and Phe44Leu; LC MS/MS identified the Val122Ile mutant peptide but failed to identify Phe44Leu. Another patient was homozygous for the Val122Ile mutation; this mutant peptide was identified by LC MS/MS, but it could not discern homozygosity. The remaining 5 patients were heterozygous and had one of the following mutations: Ala19Asp, Ile68Leu, or Val122Ile.

DISCUSSION & CONCLUSIONS: Genetic testing is an important piece in amyloid subtyping and should not be omitted in most cases indicating transthyretin amyloidosis. While LC MS/MS and proteomics have a high sensitivity and specificity, this technology cannot differentiate between homozygous and heterozygous individuals. Additionally, we have shown it did not detect a mutation in 14% of cases. Correct recognition of hereditary transthyretin amyloidosis is important for estimating prognosis, for proper familial counselling, and for guiding use of therapies, such as liver transplantation and perhaps eligibility for newer medications.

REFERENCES:


Table 1. List of mutations missed by LC MS/MS and the number of patients in whom it was not identified.

<table>
<thead>
<tr>
<th>Mutation (HGVS Standard Nomenclature)</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.Ala19Asp (p.Ala39Asp)</td>
<td>1</td>
</tr>
<tr>
<td>p.Phe44Leu (Phe64Leu)</td>
<td>1</td>
</tr>
<tr>
<td>p.Ile68Leu (p.Ile88Leu)</td>
<td>1</td>
</tr>
<tr>
<td>p.Val122Ile (p.Val142Ile)</td>
<td>4*</td>
</tr>
</tbody>
</table>

* One patient was homozygous.
Therapeutic effect of polyamidoamine dendrimer on transthyretin-related amyloidosis.

H Jono¹,², T Anno³, Y Hayashi³, K Motoyama³, M Ueda⁴, K Obayashi⁵, H Arima³, Y Ando⁴

¹ Department of Pharmacy, Kumamoto University Hospital, Kumamoto, Japan. ² Department of Clinical Pharmaceutical Sciences, ³ Department of Physical Pharmaceutics, Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan. ⁴ Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan. ⁵ Department of Morphological and Physiological Sciences, Graduate School of Health Sciences, Kumamoto University, Kumamoto, Japan.

hjono@fc.kuh.kumamoto-u.ac.jp

INTRODUCTION:

Transthyretin (TTR)-related familial amyloidotic polyneuropathy (FAP) induced by amyloidogenic TTR (ATTR), is characterized by systemic accumulation of amyloid fibrils. TTR is a tetrameric protein associated with FAP caused by tetramer dissociation and monomer misfolding. It has been proposed that structural changes within the monomer caused by protein misfolding are rate-limiting step to form TTR amyloid fibril aggregation. However, no effective therapy targeting this step is available at present. Starburst polyamidoamine dendrimers (Dens) is a spherical, highly ordered, and dendritic polymer with positively charged primary amino groups on the surface. Recent studies have shown that Dens may bind to amyloid structures and prevent the amyloid protein aggregation. In this study, we examined the effect of Dens on TTR amyloid formation and evaluated its application possibility of novel therapeutic tool for FAP.

MATERIAL & METHODS:

Both WT-TTR and ATTR-V30M were purified from serum samples obtained from healthy volunteers and homozygotic FAP ATTR V30M patients, respectively. To assess the effect of Dens on TTR amyloid formation in vitro, thioflavin T fluorimetric assay was performed. The presence of TTR amyloid fibrils was confirmed by electron microscopic analysis. To evaluate the effect of Dens on the conformational change of TTR, Far-UV circular dichroism (CD) spectra analysis was performed. We also evaluated the in vivo effect of Dens on TTR deposition in transgenic rats possessing a gene encoding human ATTR V30M (ATTR V30M Tg rats), an existing useful animal model of FAP.

RESULTS:

The amyloid formation of both WT-TTR and ATTR-V30M were suppressed by Den (G2) in a dose- and time-dependent manner. In the presence of Den (G2), no significant TTR amyloid fibrils were detected by electron microscopic analysis. CD spectra analysis showed that Den (G2) reduced the conformational change of TTR in the process of amyloid formation. Moreover, in ATTR V30M Tg rats, TTR deposition in the colon was significantly reduced by Den (G2) treatment.

DISCUSSION & CONCLUSIONS:

Those data suggest that Den (G2) may modify the stability of TTR conformation by molecular interactions, which, in turn, leads to the suppression of TTR amyloid formation. From a practical application perspective, Dens may have the potential to become a novel curative medicine for TTR-related amyloidosis.
INTRODUCTION: Delayed diagnosis can negatively impact the prognosis of patients with cardiac amyloidosis (both AL and ATTR amyloidosis), yet clinicians may not consider amyloid in their assessments because of the rare nature of both diseases. Cardiac amyloidosis is widely known as the “great imitator” due to the overlap of initial symptoms with those of many common conditions. At the 2016 American College of Cardiology annual meeting, the Amyloidosis Research Consortium (ARC) convened a roundtable to discuss medical education opportunities for the cardiology community and to establish an algorithm for the suspicion and diagnosis of cardiac amyloidosis.

MATERIAL & METHODS: Participating were five cardiologists, two hematologists, eight cardiology fellows, one nurse practitioner, one doctor of osteopathic medicine, one internist, three representatives from ARC, and five representatives from the biotechnology industry. During the breakout sessions, participants were divided into four groups. Each group was assigned a unique topic (diagnosis or medical education of AL or ATTR amyloidosis, respectively). Each group compiled a list of proposed initiatives. Participants voted on the most impactful or important idea from each group, and all convened to discuss.

RESULTS: The two top-rated ideas from each breakout group are described. For AL education, it was recommended that a registry of AL patients be developed and administered through ARC and that guidelines for evaluation—including FLC testing—of heart failure (HF) patients be developed. For ATTR education, it was recommended that more Grand Rounds outside of medical congresses be held to educate cardiologists about ATTR and that educational initiatives be expanded to include primary care providers, physician assistants, nurse practitioners, and radiologists. The two top-rated suggestions for earlier AL diagnosis were free light chain testing for all HF patients and routine strain analysis with wall thickness assessment of HF patients. Finally, for earlier ATTR diagnosis, the two top-rated recommendations were to develop a diagnostic algorithm including PYP scanning and to implement a PYP training program.

DISCUSSION & CONCLUSIONS: This discussion representing a diversity of treatment centers, industry partners, and medical disciplines resulted in diverse ideas and a commitment for ongoing collaborations to implement initiatives. Professional meetings such as ISA could provide additional opportunities to generate ideas and align with more clinicians to produce tangible improvements in awareness and patient care.
TTR quality control system in stem cell derived hepatic cells obtained from FAP patients

A Zibert¹, L Fleischhauer¹, C Niemietz¹, S Guttmann¹, V Sauer¹, HH Schmidt¹

¹Klinik für Transplantationsmedizin, Universitätsklinikum Münster, Münster, Germany.

hepar@ukmuenster.de

INTRODUCTION: A decreased thermodynamic stability of TTR tetramers is thought to induce the pathogenic process of TTR-related amyloidosis as suggested by previous findings, mostly using recombinant TTR purified from E.coli. Following translation of TTR in the cell, a competition between ER-assisted folding (ERAF) and ER-associated degradation (ERAD) seems to be an important parameter of disease. One growing current hypothesis of TTR-related disease therefore links the pathogenic mechanism to the protein quality control system. Some major genes were already characterized, including PFDN6, DNAJC7, FKBP2 and ERP29, all of which are chaperones associated with the ER and extracellular co-chaperones. Most of the previous findings on the TTR quality control system have been achieved by ectopic TTR expression in commercial cell lines that are not related to disease. Also, the genuine genetic background of FAP patients was previously not assessed.

MATERIAL & METHODS: A platform of induced pluripotent stem cells (iPSC) obtained from patients having various degrees of familial amyloidotic polyneuropathy (FAP) was generated using transient expression vectors. The iPSCs were characterized by RT-PCR, immunostainings, and functional analysis. iPSC were differentiated into hepatocyte-like cells (HLCs). Tafamidis, the only approved drug in the EU for stabilization of the TTR tetramer, was also analyzed in these cells. Gene expression as well as the presence of different forms of TTR found in the cells and in cellular supernatants were assessed.

RESULTS: Expression of Oct3/4, Nanog, TRA-1-60 and SSEA-4 was verified in iPSC derived from FAP patients by immunofluorescent staining. Immunostainings of HLCs show expression of hepatic markers albumin, HNF4a and TTR. TTR mRNA was highly expressed in HLCs as assessed by RT-PCR analysis. Various forms of TTR (monomers, dimers, tetramers) could be observed in the supernatant and cellular lysates by Western blot analysis. RT-PCR analysis of a set of 22 marker genes related to protein quality control revealed high expression in HLCs.

DISCUSSION & CONCLUSIONS: The data suggest that iPSC-based cells derived from patients having TTR-related amyloidosis are an excellent model to study patient-specific disease mechanisms, including the role of the protein quality control system.
**PA118**

**FAP renal epithelial cells isolated from urine can be reprogrammed into induced pluripotent stem cells**

V Sauer¹, C Niemietz¹, J Stella¹, G Chandhok¹, S Guttmann¹, A Zibert¹, HH Schmidt¹

¹Klinik für Transplantationsmedizin, Universitätspoliklinik Münster, Münster, Germany.

hepar@ukmuenster.de

**INTRODUCTION:** Mutations of the gene transthyretin (TTR) cause familial amyloid polyneuropathy (FAP), a neurodegenerative disease which is characterized by symptoms affecting the heart and the peripheral nervous system. More than 100 TTR mutations are currently known, leading to a misfolded TTR protein and resulting in extracellular tissue deposition. TTR is mostly (>95%) expressed by the liver. Human hepatocytes derived from somatic cells of FAP individuals would be useful in developing cell-based disease models, drug development and regenerative medicine. Although several types of somatic cells have been reprogrammed to induced pluripotent cells (iPSCs) and then differentiated to hepatocyte-like cells (HLCs), the method for generating such cells from FAP renal epithelial cells shed in human urine has not been described yet.

**MATERIAL & METHODS:** Fresh urine (250-500ml) was collected from 22 FAP patients and 12 control individuals. The washed cell pellets were expanded in cell culture in a defined growth medium. The cultivated urinary cells were characterized by immunocytochemistry using specific antibodies. iPSC reprogramming was performed using a non-transgene integrating method that delivers the pluripotency factor genes OCT3/4, SOX2, KLF4 and MYC by nucleofection of episomal (EBNA) plasmids. After characterization of stable FAP iPS cell lines, a 3-step differentiation protocol toward hepatocytes was initiated by the addition of specific growth factors (activin A, Wnt3a, FGF2, HGF). The expression pattern of definitive endoderm and hepatocyte marker genes was assessed by qRT-PCR. Flow cytometry, immunocytochemistry and hepatocyte-specific functional assays were performed.

**RESULTS:** After 2 weeks of cultivation of urinary cells, stable cell populations emerged which were positive for characteristical mesenchymal stem cell (MSC) marker and the mineralocorticoid receptor (MCR). Reprogramming of the renal epithelial cells yielded iPSCs with characteristic features. Hepatic differentiation of iPSCs generated cells expressing hepatocyte markers such as AAT, AFP, albumin, APOA1, ASGPR1, SULT2A1 and TTR, as shown by qRT-PCR. Immunocytochemistry indicated a positive cell staining for albumin, TTR, HNF4α and AFP. Flow cytometry revealed 80% of human serum albumin positive cells. Moreover, the HLCs exhibited glycogen storage.

**DISCUSSION & CONCLUSIONS:** Cells that shed from the renal epithelial system into the urine seem to be ideal targets for reprogramming into induced pluripotent stem cells (iPSCs) due to their noninvasive origin. Urine cell-derived iPSCs can be reprogrammed and then efficiently differentiated to HLCs. Our methods allowed the expression of liver specific functions. Thus, urine is a readily available source for generating FAP hepatocyte-like cells that could be potentially useful for disease modeling, pharmacological development and regenerative medicine.
PA119

Diagnosis and treatment of senile systemic amyloidosis - single center experience

T Pika1, P Flodrova2, J Vymetal3, J Minarik1, V Scudla1

1Department of Hematooncology, University Hospital Olomouc, Olomouc, Czech Republic. 2Department of Clinical and Molecular Pathology, University Hospital Olomouc, Olomouc, Czech Republic. 33rd Department of Internal Medicine, University Hospital Olomouc, Olomouc, Czech Republic.

Tomas.Pika@seznam.cz

INTRODUCTION: Senile systemic amyloidosis (SSA) is caused by the deposition of molecules of native transthyretin (TTR, prealbumin). SSA is manifesting mainly as heart disease characterized by restrictive/hypertrophic cardiomyopathy with clinical symptoms of heart failure. The prevalence estimate represents approximately 25% of the autopic findings in male population > 80 years. Diagnosis and differential diagnosis depends on strict typing of amyloid type in bioptic material, exclusion of AL and hereditary types of amyloidosis caused by the deposition of mutated transthyretin. Treatment is symptomatic, specific antifibrillar therapy is currently used in clinical trials.

MATERIAL AND METHODS: The analysed group consisted of 7 patients (6 males, 1 female) with biopsy-verified senile amyloidosis (4x endomyocardial biopsy, 2 rectal and 1 lung biopsy) with advanced cardiac impairment (NYHA III-IV). The aim of our study was to analyze the clinical features and treatment outcome of our patients.

RESULTS: In 6/7 patients supraventricular arrhythmias, such as atrial fibrillation were detected, all patients had clinical symptoms of carpal tunnel. Echocardiographic examination confirmed in all patients restrictive/hypertrophic cardiomyopathy with depression of EFLV in 6/7 patients. In 6/7 patients cardiac magnetic resonance was performed, with positive results. In all patients, immunohistochemistry and proteomic examination of biopsy samples showed massive positivity for transthyretin, in all patients AL type of amyloidosis was excluded and sequencing of TTR gene excluded hereditary form of TTR amyloidosis. Patients were initiated usual symptomatic therapy and all patients started a specific treatment combination of TUDCA (tauroursodeoxycholic acid) with doxycycline. In 4 patients (all were males) treated for more than a year (12-30 months) clinical status and NYHA class improved. We also registered decrease in NT-proBNP levels and LVEF improvement. Conversely, treated female patient died of progressive disease without response to administered therapy.

DISCUSSION AND CONCLUSION: Senile systemic amyloidosis is a relatively common, but rarely diagnosed disease with dominant cardiac impairment. Differential diagnosis is one of the fundamental aspects of the disease, requiring the cooperation of various medical experts. Treatment combination TUDCA and doxycycline appears to be effective and well tolerated.

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PA120

Evolution of ophthalmological alterations in TTR Met30 FAP on tafamidis

Ferreira Natália(1), Coelho João (1) Fonseca Isabel (2), Vilas-Boas Maria (2), Coelho Teresa (3)

(1) Department of ophthalmology, Centro Hospitalar do Porto – Hospital de Santo António (2) Unidade Corino de Andrade (3) Department of Neurophysiology, Centro Hospitalar do Porto – Hospital de Santo António;

nataliferre@gmail.com

INTRODUCTION: to report the evolution of ophthalmological alterations (amyloid deposits on lens anterior capsule and on pupillary border, scalloped pupil, and vitreous opacities) in TTR-FAP patients on tafamidis.

METHODS: retrospective analysis of 43 FAP patients on tafamidis with no ocular change at baseline. The overall survival was estimated by the Kaplan-Meier method. The follow-up was calculated since the initiation of tafamidis until February, 2016. The influence of the onset age, as well as the evolution time of the disease, was analyzed in a proportional hazards Cox regression model with some ocular changes (amyloid deposits on lens anterior capsule and on pupillary border, and vitreous opacities) as the events. Kaplan-Meier analysis was used to test for sex difference in the time to ophthalmologic events.

RESULTS: 23 were female (54%) and mean onset age was 33± 9 years. Mean evolution time of the disease was 2.7± 2 years at the beginning of tafamidis therapy, and the mean follow-up time 7.3± 1.8 years. At the end of the study, amyloid deposits on anterior lens capsule were present in 17,4% of eyes, amyloid deposits on pupillary border in 14%, scalloped pupil in 3,5% and amyloid vitreous opacities in 23,3%. All this ocular changes were observed only in women.

Kaplan-Meier survival analysis demonstrated significantly higher amyloid deposits on lens anterior capsule and vitreous opacities in female patients. The higher mean onset age was statistically significant associated with a higher risk of amyloid deposits on anterior lens capsule, amyloid deposits on pupillary border, and vitreous opacities.

CONCLUSIONS: during 7.3 years follow-up on tafamidis, ocular changes like vitreous opacities, amyloid deposits on lens anterior capsule and on pupillary border were observed only in female patients, in 23,3%, 17,4% and 14% of eyes, respectively. The higher onset age was statistically significant associated with a higher risk of ophthalmological events.
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Extremely early onset hereditary ATTR amyloidosis with p.G67R (G47R) mutation

Y Kobayashi1, Y Sekijima1,2, Y Ogawa1, Y Kondo1, N Ohashi1, S-I Ikeda1,2

1Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan. 2Institute for Biomedical Sciences, Shinshu University, Matsumoto, Japan.

juriruri@shinshu-u.ac.jp

INTRODUCTION:
Hereditary ATTR amyloidosis generally develops after 20 years old and rarely occurs in the late teens. Here, we report a Japanese family with hereditary ATTR amyloidosis in which one family member developed the disease in their early teens.

MATERIAL & METHODS:
We analyzed the clinical findings of a Japanese family with hereditary ATTR amyloidosis with G47R (p.G67R) mutation and reviewed the literature concerning the same TTR gene mutation.

RESULTS:
Case 1 was a 23-year-old woman born in Tochigi Prefecture (non-endemic area of hereditary ATTR amyloidosis). She had been well until 18 years old, when she noticed sensory disturbance in the lower limbs. At age 23, she developed orthostatic hypotension, anorexia, and blurred vision, and was diagnosed as having vitreous opacity. Technetium-99m pyrophosphate (99mTc-PYP) scintigraphy showed abnormal myocardial uptake. Amyloid deposition was observed in abdominal fat aspiration biopsy and gastroduodenal biopsy. Analysis of the TTR gene identified heterozygosity for G47R mutation.

Case 2 was a younger brother of Case 1. At age 13, he noticed blurred vision and floaters, and was diagnosed as having vitreous opacity. At age 14, he developed orthostatic hypotension, weight loss, and sensory disturbance in the lower limbs. 99mTc-PYP scintigraphy performed at age 18 showed abnormal myocardial uptake. A diagnosis of hereditary ATTR amyloidosis was made based on heterozygosity for G47R mutation and amyloid deposition in abdominal fat and gastroduodenal tissue.

Case 3, the mother of Cases 1 and 2, died of cardiac amyloidosis at age 36.

DISCUSSION & CONCLUSIONS:
To date, five hereditary ATTR amyloidosis families—three Italian families1, one Japanese family2, and one Chinese family3—with the G47R mutation have been reported, and three Italian patients with this mutation developed amyloidosis in the late teens. To our knowledge, our patient, Case 2, represents the youngest patient with hereditary ATTR amyloidosis reported to date. Our patient showed that hereditary ATTR amyloidosis could develop in the early teens in patients with specific mutations. Genetic anticipation may explain the extremely early onset amyloidosis, although the precise pathophysiological mechanism of this phenomenon remains to be elucidated.

REFERENCES:
Impact of baseline neurologic score on disease progression in transthyretin familial amyloid polyneuropathy

H Li, B Gundapaneni, J Schwartz, D Keohane, L Amass

Pfizer Inc, New York, NY, USA.

INTRODUCTION: Clinicians are struggling with optimal ways to define disease progression in transthyretin familial polyneuropathy (TTR-FAP) in order to more sensitively assess patient’s response to treatment. In the tafamidis pivotal trial, disease progression was defined as a ≥ 2-point increase from baseline in the Neuropathy Impairment Scale for Lower Limbs (NIS-LL). However, a patient’s neurologic progression is not static, and the baseline level of neurologic impairment can impact disease progression. To evaluate this further, pivotal data from the placebo-controlled tafamidis clinical trial together with data from long term extension studies were used in a prediction model to assess the impact of baseline disease severity on peripheral neurologic function over time.

MATERIAL & METHODS: Data from the placebo-treated Val30Met TTR-FAP patients (n=61) of the intent-to-treat (ITT) population in the 18-month tafamidis pivotal study along with its tafamidis-treated and ongoing long term open-label extension study data were used to fit the prediction model (data cutoff: Dec 31, 2014). A linear mixed-effects model was used, with a measured value of NIS-LL at each visit as a response variable. Baseline NIS-LL, treatment group, the interaction between baseline NIS-LL and time, and the interaction between treatment group and time were fixed effects. The model included a random effect of intercept and slope for each subject over time. Treatment group included placebo and tafamidis in the pivotal study and tafamidis-tafamidis and placebo-tafamidis in the open-label studies. Time was defined as the day of first dose in the pivotal study to the day of assessment. The current work focused on an exploration of the disease trajectory of placebo-treated patients and specifically the impact of their baseline severity on their neurologic progression over time.

RESULTS: The placebo cohort was 43% male, 89% Caucasian, with a mean (standard deviation, SD) age of 38.4 (12.9) years, mean (SD) disease duration of 34.7 (32.9) months, and mean (SD) NIS-LL of 11.4 (13.5) (min=0, max=57) at baseline. The predicted mean change in NIS-LL from baseline to month 12 as a function of baseline disease severity for the placebo cohort is presented in Figure 1. At baseline NIS-LL values between 0 and 10, the projected level of NIS-LL increase was estimated to range from 2.2 to 3.9, respectively. At higher baseline values between 20 and 30, the projected level of NIS-LL increase was estimated to range from 5.7 to 7.4, respectively.

DISCUSSION & CONCLUSIONS: Defining disease progression using a fixed threshold does not take into account baseline neurologic function and its potential influence on disease progression. The results of this predictive model of neurologic progression in TTR-FAP demonstrate that disease progression strongly depends on baseline disease severity.

Familial wild-type transthyretin cardiomyopathy

MD Benson¹, JL Berk², LH Connors³

¹Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, Indiana USA, ²Amyloidosis Center, ³Department of Pathology and Laboratory Medicine, Boston University School of Medicine, Boston, Massachusetts USA

mdbenson@iupui.edu

INTRODUCTION: Transthyretin (TTR) amyloidosis was originally described as an autosomal dominant inherited systemic disease with peripheral neuropathy being the paramount feature. It was obvious from the first description that practically all organ systems could be involved with amyloid deposition and it is now recognized that cardiomyopathy may be the most significant manifestation. In addition to familial amyloidotic polyneuropathy (FAP), it was recognized that cardiomyopathy due to transthyretin amyloid deposition could also be a sporadic disease. Westermark, P., et al., demonstrated in 1990 that the amyloid fibrils of these sporadic cases were composed of normal transthyretin. This was originally called senile cardiac amyloidosis but is now designated as ATTR wild-type and has become a prevalent diagnosis in men in their 7th or 8th decades. For unknown reasons, women appear to be infrequently involved with this disease. While most cases of ATTRwt have no indication of inheritance, we now report a family in which multiple members have ATTRwt cardiomyopathy. This suggests that in some cases there may be a genetic basis for the disease.

MATERIAL & METHODS: A 70-year-old Caucasian man died seven years after identification of cardiomegaly on an incidental chest X-ray, and three years after onset of congestive heart failure. At post mortem, amyloid cardiomyopathy was confirmed. Amino Acid sequencing of isolated amyloid fibrils showed normal sequence and DNA analysis isolated from post mortem tissue showed no mutation in the TTR gene. A few years later a brother, age 82, presented with cardiomyopathy. Abdominal fat aspirate was positive for amyloid deposition which was identified as TTR by mass spectrometry. DNA sequencing revealed no mutation in exons 1-4 of the TTR gene. Further family history indicates that an older deceased brother also had amyloidosis which was not chemically characterized.

DISCUSSION & CONCLUSIONS: FAP was originally described as an autosomal dominant inherited condition and, although 100% penetrance is not present in all families, the genetic basis is not questioned. Sporadic cases of FAP in which TTR mutations are identified usually reveal an inheritance pattern when explored in more detail. To date only two cases of spontaneous mutation in TTR causing systemic amyloidosis have been published. On the other hand, ATTR wild-type is generally considered to be a sporadic disease although the predisposition for the disease in males does suggest that other factors, possibly genetic, are at play. The family described here suggests that genetic factors may be important in expression of this disease. These might involve genetic factors that are important to the catabolism of TTR or possibly tissue factors intrinsic to the heart or other specific tissues which promote amyloid fibril formation from normal transthyretin. Certainly further research is needed to understand this new finding.

REFERENCES:

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Hereditary ATTR amyloidosis in the Netherlands: cardiac disease at the time of diagnosis and age of disease onset

SHC Klaassen, BPC Hazenberg, MP van den Berg, DJ van Veldhuisen

1 Department of Cardiology, University Medical Center Groningen, Groningen, The Netherlands. 2 Department of Rheumatology and Immunology, University Medical Center Groningen, Groningen, The Netherlands.

s.h.c.klaassen@umcg.nl

INTRODUCTION:

In hereditary ATTR (transthyretin-derived) amyloidosis the clinical presentation usually determines the first medical specialty contact. This often results in a mono-disciplinary approach for clinical assessment, diagnostic tests, and follow-up. The risk of this restricted approach is delayed detection of other organ involvement in this systemic disease that affects the heart, nerves and eyes. The aim of this study was to investigate cardiac symptoms in patients with hereditary ATTR amyloidosis in The Netherlands at the time of diagnosis, irrespective of the presentation.

MATERIAL & METHODS:

In this retrospective study patients with hereditary ATTR amyloidosis were included. For inclusion patients needed to have a proven TTR mutation and a biopsy positive for amyloid. All included patients were consecutively seen at the outpatient clinic of the University Medical Center Groningen between 1994 and 2014. Baseline was set at time of diagnosis, confirmed by the first biopsy positive for amyloid. Patients were grouped based on age, gender and genotype. The presentation was predominantly cardiac, neurologic, CTS (carpal tunnel syndrome) or family (no symptoms, only family history). For each ATTR patient clinical, echocardiographic and laboratory measurements were collected. NT-proBNP plasma levels (ng/l) were used to assess the degree of cardiac involvement. Statistics were performed using the chi-square test, the Kruskal-Wallis equality-of-populations rank test, or the One-way ANOVA where appropriate. Pearson’s r was used to calculate the correlation between age and log-transformed NT-proBNP plasma levels. A p-value of less than 0.05 was deemed significant.

RESULTS:

Seventy-one patients were included in the study. Based on genotype three groups with more than 20 subjects could be distinguished: Tyr114Cys (n=21), Val30Met (n=26) and a group comprising all other genotypes (n=24). Based on age four groups were distinguished: 20-35 y (n=11), 36-50 y (n=16), 51-65 y (n=30) and 66-80 y (n=14). Slightly more males than females were present (55%) and the mean (SD) age at diagnosis was 52 (13) years. Mean age among the different genotype groups did not differ (p=0.359). Per age category most often a neuropathy was the presenting symptom: 20-35 y (64%), 36-50 y (75%), 51-65 y (80%) and 66-80 y (50%). The age at disease onset differed among the presentations: neuropathy 52 ± 12 y, cardiomyopathy 64 ± 12 y, family 37 ± 8 y and CTS 49 ± 3 y (p<0.001). In all patients a correlation was found between age and NT-proBNP plasma levels (r=0.552, p<0.001). The elevated NT-proBNP levels exceeded the expected physiological age-related increase. In the Cys114 group no correlation was observed (r=0.389, p=0.111), but this correlation was both present in the Met30 genotype group (r=0.737, p<0.001) and in the group with all other mutations (r=0.564, p=0.007). This correlation between age at onset and NT-proBNP remained present in both males (r=0.549, p<0.001) and females (r=0.533, p=0.004).

DISCUSSION & CONCLUSIONS:

The older the ATTR patients are at diagnosis, the more frequent cardiac involvement is present, irrespective of the initial presentation. Therefore, a multidisciplinary approach is the right and only way to manage and treat this systemic disease.

REFERENCES: none
Characteristics of wild-type transthyretin cardiac amyloidosis
– Removing a myth

E. González-López1,2, C. Gagliardi3, F. Dominguez4, A. Milandri3, FJ. De Haro-del Moral4,
M. Cinelli3, C. Salas5, M. Lorenzini3, L. Alonso-Pulpón4, C. Rapezzi3, P. Garcia-Pavia1,2

1Heart failure and inherited cardiac disease Unit. Department of Cardiology. Hospital Puerta de Hierro
Majadahonda, Madrid, Spain. 2Myocardial Pathophysiology Area, Centro Nacional de Investigaciones
Cardiovasculares (CNIC), Madrid, Spain. 3Institute of Cardiology, University of Bologna and S Orsola-Malpighi
Hospital, Bologna, Italy. 4Department of Nuclear Medicine, Hospital Puerta de Hierro Majadahonda, Madrid.
5Department of Pathology. Hospital Puerta de Hierro Majadahonda, Madrid, Spain.
esthgonzalez@hotmail.com

INTRODUCTION: Wild-type transthyretin amyloidosis (ATTRwt) is a poorly known and underdiagnosed entity. It is
mostly considered a predominantly male disease of the elderly, characterized by concentric left ventricular (LV)
hypertrophy2, preserved LV ejection fraction (LVEF), low QRS voltages2 and a history of carpal tunnel syndrome (CTS)3.
We sought to describe the characteristics of a large cohort of patients with ATTRwt to better define the disease.

MATERIAL & METHODS: Clinical charts of all consecutive patients diagnosed with ATTRwt at 2 university
hospitals in Madrid (Spain) and Bologna (Italy) were reviewed. ATTRwt was diagnosed invasively by demonstration of
TTR deposits at biopsy or non-invasively, in the presence of: LV hypertrophy³ ≥ 12 mm., intense uptake on ⁹⁹mTc-DPD
scintigraphy (Perugini score 2-3)⁴ and exclusion of AL amyloidosis. In all cases genetic testing demonstrated wild type
TTR.

RESULTS: The study cohort consisted of 108 patients (aged 79 ± 8 years) diagnosed with ATTRwt at Bologna (n=71)
and Madrid (n=37). 67 patients (62%) were diagnosed invasively (97% by endomyocardial biopsy), and 41 (38%) non-
invasively. 20 patients (19%) were females. Mean maximal LV wall thickness was 17.5 ± 3.5 mm and an asymmetric
hypertrophy pattern was observed in 25 patients (23%). Mean LVEF was 52 ± 14% with 39 patients (37%) showing
a LVEF<50%. Median Myocardial Contraction Fraction (MCF) was 30% (21-43). Atrial fibrillation (60 patients, 56%)
and pseudo-infarct pattern (60, 63%) were the commonest ECG findings. Only 22 (22%) patients fulfilled low QRS
voltage criteria while 10 (11%) presented LV hypertrophy on ECG. Median voltage-to-mass was 0.17mV/g/m² (0.12-
0.26). Although heart failure was the most frequent clinical profile leading to diagnosis (73, 68%), 8 (7%) individuals
presented with atrioventricular block and 12 (11%) were diagnosed incidentally in the occasion of a scintigraphy
performed for other reasons. One third of patients (36, 33%) had a history of CTS. More than a third (35, 35%) had
been previously misdiagnosed, mainly as “hypertensive cardiomyopathy”. Overall survival at 12, 24 and 36 months
was 93%, 89% and 74% respectively.

DISCUSSION & CONCLUSION: The clinical spectrum of ATTRwt is heterogeneous and partly differs from the classical
clinical picture of this disease currently accepted in the literature: women are affected in a significant proportion of
cases; asymmetric LV hypertrophy and impaired LVEF are not rare; the commonest ECG finding is a pseudo-infarct
pattern and only a minority of patients show low QRS voltages. Clinicians should be aware of the broad clinical
spectrum of ATTRwt in order to correctly identify this disease potentially amenable with new therapies under
investigation.

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The ever-growing understanding of transthyretin amyloidosis nephropathy

Carla Leal Moreira\textsuperscript{1,2}, Ana Rocha\textsuperscript{2,3}, Josefina Santos\textsuperscript{1,2}, Marta Santos\textsuperscript{2,3}, Teresa Coelho\textsuperscript{2,3}, Luisa Lobato\textsuperscript{1,2,3}

\textsuperscript{1}Department of Nephrology, Hospital de Santo António, Centro Hospitalar do Porto \textsuperscript{2}ICBAS, University of Porto, Portugal

\textsuperscript{3}Unidade Corino de Andrade, Hospital de Santo António, Centro Hospitalar do Porto

moreira.l.s.carla@gmail.com

INTRODUCTION: Hereditary transthyretin V30M (ATTR) amyloidosis (ATTR p.Val50Met) is a disease with varied phenotypes, although the identifying signs are neuropathy and cardiomyopathy\textsuperscript{1}. Massive glomerular and vascular amyloid ATTR deposits are directly related to proteinuria and progressive reduction of glomerular filtration rate (GFR) leading to end-stage renal disease (ESRD)\textsuperscript{2}. Despite the emergence of anti-amyloid agents, nephropathic patients have limited access to disease-modifying treatments.

The aim of this study was to obtain a better knowledge of characteristics and outcomes of patients with ATTR undergoing renal replacement therapy (RRT) as a tool to future answers and early detection of nephropathy.

METHODS: We conducted a retrospective study, which included all ATTR V30M patients referred and followed in our Center who started dialysis from 2012 to 2015 (n=19). Patients with other concurrent etiologies for kidney disease were excluded (n=1). The following variables were analyzed: gender, age at first onset of neuropathy, proteinuria, onset of neuropathy, nephrologist referral and admission to dialysis, blood pressure and pro-B-type natriuretic peptide (pro-BNP) when dialysis was initiated and period of time on RRT. We also conducted a retrospective review of family pedigrees.

RESULTS: Eighteen ESRD patients (3 males, 15 females) were registered; seventeen patients were on intermittent chronic hemodialysis and one on peritoneal dialysis. The median age of neuropathy onset and proteinuria identification was 50.32 (IQ 42.50-58.84) and 54.33 (IQ 44.09-61.23) years old (yo), respectively. Proteinuria preceded neuropathy onset in 72.2% of the patients with a median time of anticipation of 4.93 (IQ 3.00-8.00) years. Most patients evolved with nephrotic proteinuria (61.5%, n=8). Only three patients were medicated with tafamidis throughout the course of the disease. The time between first nephrologist visit and starting dialysis was 3.69 (IQ 1.76-7.64) years. The median estimated glomerular filtration rate at nephrologist referral was 55.36 (IQ 47.61-84.82) ml/min/1.73 m\textsuperscript{2}.

Patient’s mean age at time of first dialysis was 60.77 (IQ 54.50-69.70) years. The majority of the patients started hemodialysis with arterial-venous fistula (66.7%, n=12) and the median duration of renal replacement therapy was 1.42 (IQ 0.27-4.65) years, with a survival rate of 83.3% at 1 year and 78.8% at 2 years of RRT starting. Hypertension was present in 55.6% (n=10) of the patients and cardiac involvement was present in all patients, with a median pro-BNP predialysis of 3663 (IQ 1444.0-10187.0) pg/ml. Most patients had a positive family history of ATTR nephropathy (55.6%, n=10), and in 38.9% (n=7) of the cases another relative developed ESRD. A phenomenon of anticipation of age at onset of nephropathy was observed in eight of the ten first degree siblings with known history of ATTR, with a median time of anticipation of 20.44 (IQ 6.69-27.80) years. Men were a minority in this group (3:7). Five out of ten kindreds with known mutant TTR gene had proteinuria >30mg/g of creatinine at neurology referral, only one progressed to ESRD, but current median age of this group is 45 (IQ 36.5-50.0) yo.

CONCLUSIONS: Renal transthyretin amyloidosis should be considered even when there is past history of hypertension. Proteinuria preceded the onset of nephropathy in most patients who develop ESRD. Regardless the same genotype, a clear deviation to female gender and a renal phenotype in their families were identified. The anticipation phenomenon and the high intra-familial penetrance of nephropathy provided the rationale to early screening of albuminuria in relatives of ESRD patients and mutant gene carriers.

Baseline Characteristics Predict the Presence of Amyloid on Endomyocardial Biopsy Among Patients with Heart Failure and Normal Ejection Fraction

Van-Khue Ton, MD PhD1,2, Aditya Bhonsale, MD1, Nisha A. Gilotra, MD1, Marc K. Halushka, MD PhD3, Charles Steenbergen, MD PhD3, Ryan J. Tedford, MD1, Ilan Wittstein, MD1, Stuart D. Russell, MD1, Kavita Sharma, MD1, and Daniel P. Judge, MD1

1. Johns Hopkins University, Division of Cardiology, Baltimore, MD, USA
2. Columbia University, Division of Cardiology, New York, NY, USA
3. Johns Hopkins University, Department of Pathology, Baltimore, MD, USA

Recent studies have shown that TTR amyloidosis is poorly recognized among people diagnosed with heart failure with preserved ejection fraction (HFpEF). We performed a retrospective analysis of individuals with HF and normal EF referred for endomyocardial biopsy (EMB) at a single center. Over 15 years, 1199 diagnostic EMBs were performed without prior cardiac transplantation. In this cohort, 295 had normal EF, of which 259 also had heart failure and adequate data for analysis. Seventy-three patients (28%) had cardiac amyloid (CAm). Multivariable independent predictors of CAm were: older age, BMI < 30 kg/m2, peripheral neuropathy, low Sokolow-Lyon index (≤ 15 mm), increased interventricular septal thickness (≥ 1.4 cm), and EF < 75%. Mean follow up was 2.6 ± 3.3 years. CAm patients had worse survival than those without (1.5 years vs. 6.3 years, log rank p < 0.0001). CAm was an independent predictor of mortality (hazard ratio 3.21, 95% CI 1.89-5.45, p < 0.0001). The complication rate for EMB was low (2.7%), with rare pericardial effusions and arrhythmias.

Conclusions: In a cohort with HF and normal EF referred for diagnostic EMB, CAm is an independent predictor of mortality. Clinicians should be suspicious of CAm in patients with heart failure, normal EF, age over 50 years, with BMI < 30 kg/m2, peripheral neuropathy, Sokolow-Lyon index ≤ 15 mm, septal wall thickness ≥ 1.4 cm, and EF < 75%.

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ISA INTERNATIONAL SOCIETY OF AMYLOIDOSIS 2016
An aggressive form of transthyretin amyloidosis

N Dasgupta¹, AK Wang², J Hardwick³, MD Benson¹

¹Department of Cardiology, Indiana University School of Medicine, Indianapolis, Indiana USA
²Department of Neurology, University of California-Irvine, Orange, California USA
³Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, Indiana USA

mdbenson@iupui.edu

INTRODUCTION: In 1990, Ueno, et al., identified a new transthyretin (TTR) mutation in a 45-year-old Japanese man with peripheral neuropathy of six-years duration. This mutation, Ser50Arg, was the result of a single nucleotide change of T→G in the third position of the codon for Ser50 (AGT→AGG). More recently we have had occasion to identify a number of patients with systemic amyloidosis (ATTR) associated with a Ser50Arg mutation, however, all of these patients share a different nucleotide exchange with AGT50AGA.

MATERIAL & METHODS: DNA was extracted from EDTA treated peripheral blood by standard methods. Nucleotide sequencing of exons 2, 3, and 4 of TTR was performed. Amplified PCR product was cleaned using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) and then subjected to asymmetric amplification using the DTCS Quick Start Kit (Beckman Coulter, Fullerton, CA, USA). Products were analyzed on a CEQ 8000 GeXP Genetic Analysis System (Beckman coulter, Fullerton, CA, USA). Evaluation of peripheral neuropathy and cardiomyopathy was the result of standard medical care.

RESULTS: All of the newly identified Ser50Arg patients are of Mexican heritage. The first patient was 39-year-old when he presented with orthostasis of two-years duration which was followed by peripheral neuropathy. A brother died at age 44 with amyloidosis and a sister at age 44 had severe peripheral neuropathy. Their father had also died at age 44 after a seven-year history of severe peripheral neuropathy. Cardiac amyloidosis was proven by endomyocardial biopsy. Liver transplantation was accomplished at age 40 but the patient showed more signs of cardiomyopathy and died at age 46. A 42-year-old Mexican woman from a different family presented with signs of peripheral neuropathy and died at age 46. A 42-year-old Mexican man was admitted to our hospital with severe congestive heart failure manifested by asities and peripheral edema. He was also found to have profound peripheral neuropathy. An echocardiogram was consistent with amyloid cardiomyopathy. Liver biopsy proved the diagnosis with vascular involvement of TTR amyloidosis. DNA analysis showed the AGT50AGA mutation. Response to treatment of congestive heart failure was prompt but stage 4 peripheral neuropathy has precluded normal daily activity. A 43-years-old Mexican man from another family presented with stage 2 peripheral neuropathy. He had a family history of four siblings dying with amyloidosis. To date he has not shown the severe restrictive cardiomyopathy that has been identified in the other Mexican patients with Ser50Arg mutation.

Three additional patients, all of Mexican origin but of unknown relationship to the patients described above, have presented to a second amyloid center with the Ser50Arg TTR mutation. Age of presentation ranged from 27- to 53-years of age. Severe peripheral neuropathy and cardiomyopathy were features of their disease.

DISCUSSION AND CONCLUSIONS: The Ser50Arg mutation as described for the original Japanese patients with AGT50AGG and the recently identified Mexican patients with AGT50AGA mutation, both with a change of Ser@Arg at position 50, appear to give a similar clinical phenotype. This mutation is associated with aggressive familial amyloidosis with a relatively early-onset and rapid progression of peripheral neuropathy and cardiomyopathy, a phenotype shared with Glu54Lys, Leu55Pro, and other mutations in the middle portion of the TTR molecule. The significance of the position of the mutation with regards to the metabolic processing of TTR which leads to fibril formation remains to be explored. The similar phenotype between patients in Japan and those of Mexican origin tends to place less emphasis on genetic profiles such as may be operative in the disparity between the Portuguese Val30Met and the later-onset Swedish Val30Met patients.
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Proteomic blood profiling in inherited (ATTR-FAC) and acquired (ATTRwt) forms of transthyretin-associated cardiac amyloidosis

GG Chan1, CM Koch1,2, LH Connors1,2

1Alan and Sandra Gerry Amyloidosis Research Laboratory in the Amyloidosis Center and 2Department of Pathology and Laboratory Medicine, Boston University School of Medicine, Boston, Massachusetts, USA.
gechan1@bu.edu

INTRODUCTION: ATTRm (inherited) and ATTRwt (acquired) amyloidoses are fatal protein misfolding diseases that frequently feature cardiomyopathy. An accurate diagnosis of ATTR amyloidosis can be challenging as biopsy evidence, usually from the affected organ, is required. The discovery of disease biomarkers could aid in ATTR identification and potentially reveal important clues about the pathophysiological mechanisms that signal onset and progression of amyloid disease. The aim of this study was to investigate the presence of serum proteomic identifiers in ATTR cardiac amyloidosis.

MATERIAL & METHODS: Sera were from patients with ATTRwt or ATTRm featuring familial amyloid cardiomyopathy (ATTR-FAC), and controls; all groups were age-, gender-, and race-matched. ATTR cases underwent genotyping of TTR exons 1-4 and had biopsy proof of TTR amyloidosis. Serum proteomic analyses (192 proteins) were performed using the PeptiQuant™ Human Discovery Assay (MRM Proteomics, Inc.). Comparisons were made using the Welch t-test for unequal variance and data adjustment using the false discovery rate (FDR) test; significance was defined as \( p < 0.05 \). Protein levels in ATTRwt and ATTR-FAC significantly different from control data were further analyzed using STRING to predict protein to protein associations.

RESULTS: We found significant concentration differences between patient and control sera for the majority (123/192) of proteins under investigation, including TTR and retinol-binding protein (RBP4). In ATTR-FAC, serum levels of 14 proteins were identified as unique to that group (Table 1, Figure 1a) and generally lower than controls; moreover, the concentrations of RBP4 and 6 other proteins in this group were significantly different compared to ATTRwt (Table 1 *).

DISCUSSION & CONCLUSIONS: Proteomic analyses of ATTR and control sera showed significant concentration differences in 64% of the serum proteins that were studied. In comparing ATTR-FAC to ATTRwt, significant differences in serum levels, interactions and functions of several proteins, including RBP4, were found. These data suggest that the serum proteomes in ATTR and healthy age-matched controls are dissimilar. Unique differences between ATTR-FAC and ATTRwt indicate that these data may be useful in identification of ATTR and differentiating between the inherited and acquired TTR-associated cardiac amyloidosis. This project was supported by the E. Rhodes and Leona B. Carpenter Foundation Grant, National Institutes of Health ROIAG031804, and the Young Family Amyloid Research Fund.

![Fig 1. (a) Venn Diagram showing distribution of ATTR proteins different (p < 0.05) from controls. (b) STRING predicted protein interactions ATTR-FAC unique proteins.](attachment:image.png)

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Table 1. ATTR-FAC proteins (with STRING designations) that were significantly different \((p < 0.05)\) from controls. *indicates significant differences in comparisons to control and ATTRwt; RED = higher concentrations; BLUE = lower concentrations.
TRANSTHYRETIN AND RETINOL-BINDING PROTEIN SERUM CONCENTRATIONS ARE PROGNOSTIC AND SURVIVAL INDICATORS IN WILD-TYPE TRANSTHYRETIN AMYLOIDOSIS

JLS Hanson\textsuperscript{1,2}, CM Koch\textsuperscript{1,2}, GG Chan\textsuperscript{1}, T Prokaeva\textsuperscript{1}, LH Connors\textsuperscript{1,2}

\textsuperscript{1}Alan and Sandra Gerry Amyloidosis Research Laboratory in the Amyloidosis Center and \textsuperscript{2}Department of Pathology and Laboratory Medicine, Boston University, Massachusetts, USA. jisikora@bu.edu

INTRODUCTION: ATTRwt is a sporadic cardiomyopathy with an undefined pathogenesis and no validated, specific disease biomarkers. Serum TTR levels are low in ATTRm, and possibly in ATTRwt. Furthermore, non-coding genetic risk variants may affect TTR expression in ATTRwt. We aimed to determine whether serum TTR and RBP4 could be specific diagnostic and prognostic indicators in ATTRwt.

MATERIAL & METHODS: ATTRwt (n = 97), ATTRm (n = 30), and healthy control (n = 30) subjects were males over age 60; disease groups had biopsy-proven TTR amyloidosis featuring cardiac involvement, defined as inter-ventricular septal thickness (IVST) ≥ 12 mm and serum b-type natriuretic peptide (BNP) > 100 pg/mL. The ATTRm group included TTR-V122I, -T60A, and -V30M. Clinical and laboratory data at initial evaluations, along with ATTRwt follow-ups at 1-year (n = 29) and 2-years (n = 8) from baseline were obtained from the Boston University Amyloidosis Center IRB-approved clinical database. Serum TTR levels were determined by immunoturbidity in the Boston Medical Center Pathology Laboratory; serum RBP4 levels were determined by ELISA developed in our laboratory. Correlations between laboratory parameters and TTR or RBP4 were determined by Pearson or Spearman tests, depending on normality via the Shapiro-Wilk test. The Cox proportional hazard survival model was used to analyse the data.

RESULTS: Baseline serum TTR concentrations trended lower in ATTRwt compared to controls, though the difference was not significant (\textbf{Fig 1a}); however, in ATTRm, serum TTR was significantly decreased compared to all other groups (p < 0.0001). Serum RBP4 concentrations were higher in ATTRwt compared to ATTRm (p = 0.0014) and controls (p < 0.0001) (\textbf{Fig 1b}). In ATTRwt only, TTR was correlated with BNP (r = -0.21, p = 0.036) and low TTR was associated with arrhythmia (p = 0.0051), while RBP4 was correlated with cardiac troponin (cTn-I) (r = 0.22, p = 0.026) and IVST (r = 0.20, p = 0.046). Moreover, predictors of survival found only in ATTRwt were RBP4 (p = 0.016), cTn-I (p < 0.0001), and serum uric acid (p = 0.0075). In ATTRwt subjects treated with TTR stabilizers, diflunisal or tafamidis, higher serum TTR concentrations were observed at 1-year (n = 11) and 2-year (n = 3) follow-ups (\textbf{Fig 1c}) compared to untreated (n = 18, 5); the increased TTR levels in treated vs. untreated were significant at both follow-ups (p < 0.0001). Treated subjects over 2 years had increased serum TTR (p = 0.034), as well as decreased BNP (p = 0.024) and IVST (p = 0.047). In contrast, untreated subjects exhibited decreased levels of serum TTR (p = 0.014), elevated BNP (p = 0.031) and cTn-I (p = 0.0090), and decreased left-ventricular ejection fraction (LVEF) (p = 0.040) over 2 years.

DISCUSSION & CONCLUSIONS: This study identified serum TTR and RBP4 concentrations as markers of disease progression and survival in ATTRwt, but not ATTRm. Low TTR and high RBP4 levels were associated with poor disease characteristics and were predictors of survival in multivariate analysis. Further, in treated ATTRwt subjects, increased TTR levels corresponded to measurable BNP and IVST improvement, contrasting with a decrease in TTR levels along with signs of disease progression in untreated subjects.

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PB17

Long-term treatment with diflunisal for hereditary transthyretin amyloidosis – the Swedish DFNS01 experience

Jonas Wixner¹, Intissar Anan¹, Björn Pilebro¹, Hans-Erik Lundgren¹ and Ole B Suhr¹
¹Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

Background

The non-steroidal anti-inflammatory drug diflunisal has been shown to stabilize the transthyretin tetramer and thereby prevent amyloid fibril formation. As the international randomized, double-blind, placebo-controlled diflunisal trial [1] was closed for enrollment in 2011, the Swedish DFNS01 extension study was started to continue monitoring the effect of diflunisal in patients with hereditary transthyretin amyloid (hATTR) amyloidosis.

Materials and methods

DFNS01 was an open-label observational study designed to monitor the effect of diflunisal 500 mg daily on neurological impairment, cardiac involvement and nutritional status in hATTR amyloidosis and was open for enrollment from July 2011 to the publication of the outcome of the controlled diflunisal trial. The primary outcome measure was changes in the Kumamoto scale, and secondary outcome measures were changes of the nutritional status (modified body mass index, mBMI), cardiac function (septal thickness and pro-BNP) and safety follow-up blood tests (hemoglobin, platelets, creatinine, and liver enzymes). Evaluations were performed at 12 and 24 months, respectively.

Results

Fifty-three patients were included in the study. Of those, 13 (24%) had received diflunisal prior to their inclusion in DFNS01, with a median pre-study treatment of 2 (range 0.1-4) years, and 26 (49%) had completed the 24-month study follow-up. The main reasons for early termination were liver transplantation (32%), study closure (21%) and side effects from the study drug (14%). For those who had completed the study protocol, total Kumamoto score had remained stable over time (median score 13 vs. 16 vs. 17.5, p = 0.21), as had the sub-scores for sensory neuropathy, autonomic neuropathy and organ dysfunction. However, an increase in motor neuropathy scores was found (0 vs. 2.5 vs. 4.5, p = 0.02), and there was a slight decrease in nutritional status after 12 months (mBMI 1019 vs. 952, p <0.01), but not after 24 months (952 vs. 1022, p = 0.80). No significant change was found in pro-BNP levels (532 vs. 412 vs. 457 ng/l, p = 0.19), whereas cardiac septum thickness had increased over time (16.5 vs. 16.5 vs. 18 mm, p = 0.01). No significant changes were found in the safety follow-up blood tests. Sub-group analyses on the eight patients who had completed the DFNS01 protocol, and had received pre-study treatment with diflunisal in the controlled trial or by off-label prescription, revealed no significant changes in any of the outcome measures. Median duration of diflunisal treatment in this sub-group was 3.5 (2.4-5.4) years. No major differences in outcome measures were found for patients with late onset, male sex or a polyneuropathy disability score of more than II at inclusion.

Discussion and conclusions

Although limited by high dropout rates, mainly due to liver transplantation, the DFNS01 trial supports the safety and efficacy of diflunisal for hATTR amyloidosis, and the results are in line with the previous placebo-controlled trial. Total Kumamoto scores, nutritional status and blood tests had remained stable over time (in some cases for up to five years), however, motor neuropathy scores and cardiac septum thickness had increased significantly during the study, which suggests that complete disease stabilization is not achieved on group level. Further studies are needed to evaluate the long-term effect of diflunisal and to investigate whether all sub-groups of patients have the same beneficial effect from the treatment.

References

A health-related quality of life comparison of symptomatic transthyretin amyloidosis subjects and asymptomatic carriers from the Transthyretin Amyloidosis Outcomes Survey

I Conceição1, M Stewart2, M Sultan2,3, R Mundayat2, M-L Ong2, D Keohane2

1 CHLN-Hospital Santa Maria, Lisbon, Portugal. 2 Pfizer Inc, New York, NY, USA. 3 New York Eye and Ear Infirmary, New York, NY, USA.

imsconceicao@gmail.com

INTRODUCTION: Transthyretin (TTR) amyloidosis is a rare, fatal disorder characterized by deposition of amyloid in tissues with significant effects on neurologic, autonomic, and cardiac systems. The disease strikes adults in the prime of their lives. Besides the physical effects, the disease has significant impact on an affected individual’s health-related quality of life (HRQoL). This analysis compares the HRQoL in subjects with symptomatic TTR amyloidosis versus asymptomatic gene carriers.

MATERIAL & METHODS: The Transthyretin Amyloidosis Outcomes Survey (THAOS) is the largest, ongoing, international, non-interventional study of TTR amyloidosis and currently includes over 3000 subjects with both symptomatic and asymptomatic disease. The EuroQol-5 Dimensions Health Questionnaire-3 Levels version (EQ-5D) is a validated self-administered questionnaire used to measure HRQoL. EQ-5D is collected for subjects participating in THAOS, and data (cutoff date: January 14th, 2016) were analyzed to compare baseline EQ-5D index scores for a cohort of 1859 subjects with TTR mutations (1330 symptomatic subjects and 529 asymptomatic carriers). Subjects were grouped based on age at baseline and Student’s t-test was used to compare EQ-5D index scores for symptomatic versus asymptomatic carriers, and to compare each of these groups to an age-matched nationally representative United States (US) survey sample serving as controls.

RESULTS: Symptomatic TTR amyloidosis subjects had lower EQ-5D index scores than the age-matched asymptomatic carrier comparator groups from THAOS. The difference was statistically significant across all age groups (Table 1). Compared with age-matched comparator groups from the US survey, the symptomatic TTR amyloidosis subjects had lower EQ-5D index scores which were statistically significant across all age cohorts. The asymptomatic carrier cohort from THAOS had similar EQ-5D index scores for all age-matched US controls. There were no statistically significant differences between the asymptomatic carriers and the US controls for any of the age groups. EQ-5D index scores for symptomatic subjects in the 50-64 year age group were numerically lower than those reported by patients with serious conditions such as diabetes and breast cancer, but comparable to those with rheumatoid arthritis, emphysema, stroke [1].

DISCUSSION & CONCLUSIONS: We present an analysis of the impact of TTR amyloidosis on HRQoL, as measured by the EQ-5D index score, on 1859 symptomatic and asymptomatic subjects from the THAOS non-interventional study. HRQoL for symptomatic subjects was significantly lower than for asymptomatic carriers and age-matched US controls. Asymptomatic subjects had similar HRQoL to controls. The adverse effects on HRQoL were comparable to other serious conditions. Beyond the devastating impact on physical functioning, symptomatic TTR amyloidosis has a significant impact on HRQoL.


Table 1. Mean EQ-5D index score at enrollment by age group among symptomatic vs. asymptomatic subjects in the THAOS registry.

<table>
<thead>
<tr>
<th>Age Group (years)</th>
<th>Mean (SD) EQ-5D Index Score</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-34</td>
<td>Mean ± SD (n)</td>
<td></td>
</tr>
<tr>
<td>Symptomatic (n = 1330)</td>
<td>0.81 ± 0.172 (341)</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic (n = 529)</td>
<td>0.94 ± 0.101 (309)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>35-49</td>
<td>Mean ± SD (n)</td>
<td></td>
</tr>
<tr>
<td>Symptomatic (n = 447)</td>
<td>0.76 ± 0.193 (447)</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic (n = 152)</td>
<td>0.91 ± 0.140 (152)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>50-64</td>
<td>Mean ± SD (n)</td>
<td></td>
</tr>
<tr>
<td>Symptomatic (n = 292)</td>
<td>0.69 ± 0.240 (292)</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic (n = 55)</td>
<td>0.86 ± 0.139 (55)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>65+</td>
<td>Mean ± SD (n)</td>
<td></td>
</tr>
<tr>
<td>Symptomatic (n = 250)</td>
<td>0.69 ± 0.231 (250)</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic (n = 13)</td>
<td>0.83 ± 0.199 (13)</td>
<td>0.0346</td>
</tr>
</tbody>
</table>

SD, Standard deviation
Rationale and design of the Phase 3 Transthyretin Amyloid Cardiomyopathy Tafamidis clinical trial

MS Maurer1, P Elliott2, G Merlini3, SJ Shah4, M Waddington Cruz5, A Flynn6, B Gundapaneni7, C Hahn6, S Riley8, J Schwartz8, MB Sultan9,10, C Rapezzi11 on behalf of the 1028 Study Investigators

1 Columbia University College of Physicians and Surgeons, New York, USA. 2 University College London, London, UK. 3 University Hospital Policlinico San Matteo, Pavia, Italy. 4 Northwestern University, Chicago, IL, USA. 5 Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. 6 Pfizer Inc, Collegeville, PA, USA. 7 inVentiv Health, Burlington, MA, USA. 8 Pfizer Inc, Groton, CT, USA. 9 Pfizer Inc, New York, NY, USA. 10 New York Eye and Ear Infirmary, New York, NY, USA. 11 University of Bologna, Bologna, Italy.

msm10@cumc.columbia.edu

INTRODUCTION: Transthyretin cardiomyopathy (TTR-CM), a progressive and usually fatal disorder, is caused by deposition of amyloid fibrils of either mutant or wild-type TTR in the heart. At present, there are no therapies shown to improve symptoms or survival in TTR-CM. Tafamidis is a small molecule that binds selectively to TTR and slows amyloid fibril formation in vitro. The Phase 3 ATTR-ACT study (NCT01994889) is an international, multicenter, double-blind, placebo-controlled, randomized trial that evaluates the efficacy, safety, and tolerability of tafamidis (20 mg or 80 mg orally once daily) in comparison with placebo for the treatment of TTR-CM. The rationale and design of the ATTR-ACT trial are described here.

MATERIAL & METHODS: Major inclusion criteria were: confirmed diagnosis of TTR-CM with presence of amyloid deposits in biopsy tissue; age ≥18 to ≤90 years; history of heart failure (HF) evidenced by at least one prior hospitalization for HF (without hospitalization) requiring diuretics; 6-Minute Walk Test (6MWT) of >100 meters; and plasma NT-proBNP concentration ≥600 pg/mL. These inclusion criteria were chosen to ensure that patients at an early disease stage were included as tafamidis treatment has been shown to slow disease progression in patients with early stage TTR familial amyloid polyneuropathy [1,2]. Exclusion criteria include: diagnosis of light chain amyloidosis; previous treatment with tafamidis; glomerular filtration rate <25 mL/min/1.73 m2; concurrent treatment with non-steroidal anti-inflammatory drugs, calcium channel blockers, or digitalis; and modified-body mass index of <600 kg/m2•g/L. The enrollment target was approximately 400 patients, randomized to the 3 arms in a 2:1:2 ratio (placebo: tafamidis 20 mg: tafamidis 80 mg). Target enrollment numbers were increased to mitigate risk of design assumption uncertainty. Patients were stratified by baseline NYHA classification and variant or wild-type TTR, with intent to enroll comparable numbers of patients but with a minimum of 30% variant and wild-type in each group. The primary analysis uses a hierarchical combination of all-cause mortality and frequency of cardiovascular-related hospitalizations, defined as the number of times a patient is admitted to a hospital for cardiovascular-related morbidity in the 30 months following enrollment. This method (Finklestein-Schoenfeld) increases the sensitivity and power of the analysis while also preserving the importance of the all-cause mortality endpoint. For a significance level of 0.05 (two-sided test), a sample size of 300 (n=120 placebo, n=60 tafamidis 20 mg, n=120 tafamidis 80 mg) yields a power over 90%, which assumes pooling of the tafamidis dose groups. Secondary and exploratory analyses will include the changes from baseline in the 6MWT, the Kansas City Cardiomyopathy Questionnaire Overall Summary score, and echocardiographic and biomarker indices. Safety and tolerability of tafamidis will also be evaluated.

DISCUSSION & CONCLUSIONS: This trial is the largest multicenter investigation of a treatment for TTR-CM, a condition associated with high mortality and symptom burden for which there are no approved disease-modifying pharmacotherapies. This study will provide important insight into the efficacy and safety of tafamidis for the treatment of TTR-CM.

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Characterizing the high disease burden of transthyretin amyloidosis for patients and caregivers

M Stewart¹, S Shaffer², B Murphy³, J Loftus¹, J Alvir¹, M Cicchetti¹, WR Lenderking²

¹ Pfizer Inc, New York, NY, USA. ² Evidera, Bethesda, MD, USA.
michelle.stewart@pfizer.com

INTRODUCTION: Transthyretin (TTR) amyloidosis is a progressively debilitating and ultimately fatal rare disease that requires caregiver support. Little research has been conducted on the burden of disease (BOD) for TTR amyloidosis patients and their caregivers. The current study aimed to better characterize the BOD of TTR amyloidosis.

MATERIAL & METHODS: Patients and caregivers in the United States (US) and Spain were recruited through patient advocacy groups to complete a cross-sectional survey. Respondents included 60 TTR amyloidosis patients (n=44 in US; n=16 in Spain) with both polyneuropathy and cardiac phenotypes, and 32 caregivers (n=24 in US; n=8 in Spain). Assessments included the 12-Item Short Form Health Survey (SF-12), Work Productivity & Activities Impairment scale, Zarit Burden scale, pain and symptom measures, healthcare resource use, and caregiving burden questions.

RESULTS: Patients. US patients were older compared with the Spanish patients (61.7 vs. 49.3 years, respectively), and included a lower percentage who had undergone liver transplantation (38.6% vs. 87.5%). Nearly all patients in Spain had the Val30Met mutation whereas those from the US had predominantly non-Val30Met mutations. Age at symptom onset was lower for patients from Spain (41.1 vs. 56.3 years in the US). A majority (>80%) of respondents from both countries reported a family history of TTR amyloidosis. Scores as much as two standard deviations below normal were seen for physical health, and impairment was reported by patients in their quality of life, pain, and work productivity. A high rate of healthcare utilization was reported in the US and Spain over the three months prior to the survey, including ER visits (22.7% and 18.8%, respectively) and hospitalizations (15.9% and 12.5%, respectively).

Caregivers. Caregivers were primarily female, with similar mean ages in the US and Spain (57.0 and 52.8 years, respectively), and were most often spouses of the patient. Caregivers reported substantial burden due to caregiving, including poor mental health, work impairment, and time spent providing care.

DISCUSSION & CONCLUSIONS: TTR amyloidosis is associated with high levels of impairment in many domains. For patients there was a greater impact on physical than mental health, especially for those who had not undergone liver transplantation. Pain and other symptoms were likewise adversely affected. Providing care for a patient with TTR amyloidosis is associated with a negative impact on mental health as well as on many aspects of the caregiver’s life, such as relationships and social/family life. Zarit caregiver burden scores for US caregivers were comparable to scores observed in a study of caregivers of Alzheimer’s disease patients [1]. Work productivity was compromised for employed patients and caregivers. Managing TTR amyloidosis leads to high health care resource use. Patients who had undergone liver transplantation reported better outcomes overall. Limitations of the study include the small sample size, self-report nature of the survey, and differences between the US and Spanish subgroups. These results highlight the substantial burden of TTR amyloidosis for patients and caregivers.

Right ventricular involvement in transthyretin amyloidosis

Arvidsson S1, Henein MY1, Wikström G2, Suhr OB1, Lindqvist P3

1 Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden. 2 Department of Medical Sciences, Cardiology, Uppsala University, Uppsala, Sweden. 3 Department of Surgical and Perioperative Sciences, Umeå University, Umeå, Sweden.

INTRODUCTION

Right ventricular dysfunction is frequently encountered in different cardiomyopathies as well as in immunoglobulin light chain (AL) amyloidosis, and is described as a strong predictor of prognosis. However, RV involvement in transthyretin related amyloidosis (ATTR) has been somewhat overlooked. We therefore sought to establish the degree of RV involvement in ATTR amyloidosis and compare the findings with sarcomeric hypertrophic cardiomyopathy (HCM).

MATERIAL & METHODS

Forty-two patients with diagnosed ATTR and echocardiographic evidence of cardiac amyloidosis (cardiac ATTR), 19 ATTR patients with normal left ventricular (LV) wall thickness (non-cardiac ATTR), 25 patients with diagnosed sarcomeric HCM and 30 healthy controls were included in the study. Echocardiographic examinations were analysed and standard measurements of LV and RV dimensions and function were carried out. In addition, RV global and segmental strain were analysed.

RESULTS

Non-cardiac ATTR amyloidosis patients did not differ from healthy controls, either in geometrical or functional measurements of the LV and RV. When comparing cardiac ATTR and HCM patients by means of RV structure and function only segmental strain differed between the two patient groups. In cardiac ATTR we found an RV apex-to-base strain gradient with highest deformation in the apex whereas the pattern was inverse in patients with HCM (fig 1).

DISCUSSION & CONCLUSIONS

RV involvement is common only in ATTR patients with concomitant LV involvement. The present study also revealed an apical sparing pattern for the RV, which previously only has been described for the LV in patients with ATTR cardiomyopathy. This pattern was not seen in HCM patients. Further studies are warranted to assess the clinical importance of these findings.

REFERENCES


Fig. 1: Peak systolic longitudinal strain measured in basal, mid and apical regions of the right ventricle in patients with cardiac and non-cardiac transthyretin amyloidosis (ATTR), hypertrophic cardiomyopathy (HCM), and healthy controls.
SCHWANN CELL AND ENDOTHELIAL CELL DAMAGE
IN TRANSTHYRETIN FAMILIAL AMYLOID POLYNEUROPATHY

H Koike¹, S Ikeda¹, M Takahashi¹, Y Kawagashira¹, M Iijima¹,
Y Misumi², Y Ando², S Ikeda³, M Katsuno¹, G Sobue¹

¹Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan. ²Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan. ³Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan.

koike-haruki@med.nagoya-u.ac.jp

INTRODUCTION: Transthyretin (TTR) Val30Met-associated familial amyloid polyneuropathy (FAP ATTR Val30Met) is the most common form of FAP and has become prevalent in areas other than conventional endemic foci [1-3]. Although peripheral neuropathy is the cardinal feature of FAP ATTR Val30Met, its mechanism has not been fully elucidated.

MATERIAL & METHODS: We examined sural nerve biopsy specimens from 49 patients with FAP ATTR Val30Met using electron microscopy, particularly focusing on the morphology of Schwann cells and nerve microvascular endothelial cells. This study included 11 early-onset cases from endemic foci (7 men and 4 women) and 38 late-onset cases from non-endemic areas (34 men and 4 women).

RESULTS: Greater amyloid deposition was observed in early-onset cases than in late-onset cases, whereas reduced nerve fiber density was more conspicuous in late-onset cases than in early-onset cases. Atrophy of nonmyelinating Schwann cells apposed to amyloid fibrils was observed particularly in early-onset cases (Fig. 1A), indicating that direct invasion of amyloid to Schwann cells had resulted in predominantly small-fiber axonal loss characteristic in these cases [4]. On the contrary, it was not conspicuous in late-onset cases (Fig. 1B). Morphology of nerve microvascular endothelial cells was frequently found to be abnormal with loss of tight junctions and fenestrations (Fig. 1C). Abnormalities of endothelial cells were found in both early- and late-onset cases, irrespective of the presence or absence of amyloid deposition.

DISCUSSION & CONCLUSIONS: These findings suggest that, in addition to direct invasion of amyloid to Schwann cells, vasculopathy contributes to the pathogenesis of neuropathy in FAP.

Fig. 1: Representative findings of sural nerve biopsy specimens from patients with FAP ATTR Val30Met.

REFERENCES:
3 Koike H, Tanaka F, Hashimoto R, et al. Natural history of transthyretin Val30Met familial amyloid polyneuropathy: analysis of late-onset cases from non-endemic areas. J Neurol Neurosurg Psychiatry 2012;83:152-8.
Abnormal small bowel motility in patients with hereditary transthyretin amyloidosis

Wixner J¹, Törnblom H², Suhr OB¹, Karling P¹ and Lindberg G³

¹Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden, Institute of Medicine, ²Department of Medicine & Clinical Nutrition, Sahlgrenska Academy, Gothenburg University, Gothenburg, Sweden and ³Department of Medicine, Karolinska Institute, Karolinska University Hospital, Huddinge, Sweden

Background
Gastrointestinal (GI) complications such as constipation, diarrhea and gastroparesis are common in hereditary transthyretin amyloid (hATTR) amyloidosis. The mechanisms behind these disturbances have not been fully elucidated and the patients' small bowel function remains largely unexplored. The aim of the present study was to compare the small bowel motility in patients with hATTR amyloidosis with that in non-amyloidosis controls.

Materials and methods
Patients with hATTR amyloidosis undergoing evaluation for liver transplantation were consecutively investigated with 24-h small bowel manometry (n = 19) at Karolinska University Hospital, Huddinge, Sweden. Duodenojejunal recording sites were used during the tests and the somatostatin analogue octreotide was used for inducing fasting motility. The patients' GI symptoms and polyneuropathy disability (PND) score [1] had been recorded during routine clinical evaluations. Patients with an age at onset of 50 years or more were defined as late-onset cases. For each patient, three age and sex matched controls (n = 57) previously investigated with manometry due to functional GI disorders were selected.

Results
The patients' median age at examination was 52.8 (range 30.8-66.5) years and the median duration of symptomatic disease was 2.3 (0.5-9.7) years. A majority (89%) of the patients carried the V30M mutation, 58% had GI symptoms and 84% had a PND score of I (sensory disturbances but preserved walking capability). Small bowel manometry was judged to be abnormal [2] in 58% of the patients and in 26% of the controls (p = 0.01). Patients displayed significantly more phase III migrating motor complexes during daytime than the controls (median 4 vs. 2, p <0.01), and had a higher frequency of low-amplitude complexes of below 30 mm Hg (16% vs. 4%), however, this difference did not reach statistical significance (p = 0.10). Further, patients showed a delayed response to octreotide compared to controls (5.0 min vs. 3.8 min, p = 0.02). Sub-group analyses showed that late-onset cases (n = 10) had a longer delay in octreotide response (5.4 vs. 4.3 min, p = 0.03), but this was not observed for the age-matched controls (3.8 vs. 4.0 min, p = 0.63). No major differences related to sex, presence of GI symptoms, PND score (I vs. ≥II), duration of disease (≤3 vs. >3 years) or TTR mutation were found for any of the manometry variables.

Discussion and conclusions
Patients with hATTR amyloidosis, even at early stages, more frequently displayed abnormalities in their small bowel motility than patients with functional GI disorders. They also showed a delayed response to octreotide injection, which may reflect an autonomic neuropathy and changes in the neuroendocrine system of the gut. Late-onset cases had a longer delay in octreotide response, although they usually have less GI symptoms than early-onset cases, which is a finding that warrants further investigation.

References
The prognostic value of ventricular arrhythmias in transthyretin cardiac amyloidosis

AR Garan1, H Li2, B Ebede2, J Chyou1, J Dizon1, M Maurer1

1 Department of Medicine, Division of Cardiology, Columbia University, New York, NY, USA.

2 Pfizer Inc, New York, NY, USA.

arg2024@cumc.columbia.edu

INTRODUCTION: Little is known about the prevalence of ventricular arrhythmias in patients with transthyretin (TTR) cardiac amyloidosis. Prior studies have reported the prevalence of ventricular arrhythmias in patients with primary (AL) cardiac amyloidosis but findings have varied [1,2]. Furthermore, little is known about the prognostic importance of ventricular arrhythmias in this population. As such, we sought to characterize the prevalence and prognostic significance of ventricular arrhythmias in TTR cardiac amyloidosis patients.

MATERIAL & METHODS: We analyzed 24-hour Holter monitor data from patients with TTR cardiac amyloidosis to determine the prevalence of arrhythmias in this population. Patients were followed prospectively as part of NCT00694161, an on-going, open label, multicenter, single-treatment study examining the efficacy and safety of tafamidis meglumine in patients with TTR cardiac amyloidosis.

RESULTS: Thirty-four patients were included in the study and 33 had baseline Holter monitor data. Mean (SD) follow up was 3.73 ± 2.24 years. At the time of enrollment, the mean age of patients was 75.9 ± 4.6 years and 31 (91.2%) were men; thirty-two (94.1%) patients had either NYHA class I or II symptoms; mean heart rate on the 24-hour Holter monitor was 74.4 ± 7.5 beats per minute; fifteen patients (44.1%) were taking a beta-blocker, twelve (35.3%) were taking amiodarone, and three (8.8%) were taking another anti-arrhythmic medication. Non-sustained ventricular tachycardia (NSVT) was present in 20 patients (60.6%); no patients had sustained ventricular tachycardia (defined as lasting longer than 30 seconds). There was no difference in the overall survival between those with and without NSVT on 24-hour Holter monitor (p = 0.86; Fig. 1). Significant predictors of mortality during study follow up in a univariable analysis included elevated troponin I (HR 5.09; 1.61 – 16.02) and troponin T (HR 4.07; 1.55 – 10.66).

DISCUSSION & CONCLUSIONS: These findings demonstrate that ventricular arrhythmias manifested as NSVT on Holter monitor is present in the majority of patients with TTR cardiac amyloidosis. However, the presence of NSVT did not portend higher mortality in this patient population. These findings differ from observations made in patients with AL cardiac amyloidosis and may call into question the utility of implantable defibrillators in this population.


Fig 1: Survival of transthyretin cardiac amyloidosis patients with and without non-sustained ventricular tachycardia (NVST) from study NCT00694161 examining the efficacy and safety of tafamidis in patients with TTR amyloid cardiomyopathy
Transition from asymptomatic to symptomatic transthyretin familial amyloid polyneuropathy: An analysis from the Transthyretin Amyloidosis Outcomes Survey

T Coelho¹, D Keohane², M Sultan², M Carlsson², M-L Ong²

¹ Centre for the Study of Amyloidoses, Hospital Santo António, Porto, Portugal. ² Pfizer Inc, New York, NY, USA.

tcoelho@netcabo.pt

INTRODUCTION: Transthyretin familial amyloid polyneuropathy (TTR-FAP) is a rare, life-threatening disorder characterized by progressive polyneuropathy. Early diagnosis and treatment of TTR-FAP are optimal to maintain neurologic function. This analysis was performed to identify the most common symptom presentations of TTR-FAP in asymptomatic subjects converting to symptomatic disease from a global TTR-FAP disease registry.

MATERIAL & METHODS: The Transthyretin Amyloidosis Outcomes Survey (THAOS) is the largest, ongoing, international observational disease registry for transthyretin amyloidosis and currently includes over 3000 subjects with both symptomatic and asymptomatic disease. The THAOS registry dataset (dated January 14, 2016) was analyzed to identify asymptomatic subjects who converted to symptomatic TTR-FAP during follow-up. As a comparator population, all asymptomatic subjects who remained asymptomatic and who had at least one follow-up visit registered were analyzed too. The first presenting symptoms by genotype (Val30Met vs. non-Val30Met) and comparative descriptive characteristics of converters to symptomatic disease versus those remaining asymptomatic during the same time course of follow-up were evaluated.

RESULTS: In this analysis, 161 subjects converted to symptomatic TTR-FAP and 296 subjects remained asymptomatic. Data for the 161 subjects who converted to symptomatic TTR-FAP were as follows: mean age of 35.8 years, mean modified body mass index (mBMI) of 1147, gender of 36% men, 64% women and genotype of 84% Val30Met and 16% non-Val30Met. Data for the 296 subjects who remained asymptomatic were as follows: mean age of 35.6 years, mean mBMI of 1169, gender of 41% men, 59% women and genotype of 91% Val30Met, 9% non-Val30Met. Analysis of first presenting symptoms revealed gastrointestinal (GI) and sensory symptoms as the two most common presenting symptoms for Val30Met subjects compared with sensory and autonomic symptoms (other than GI) for non-Val30Met subjects.

DISCUSSION & CONCLUSIONS: This THAOS registry analysis included 457 subjects who were followed for up to almost 7 years. There were 161 subjects who converted to symptomatic TTR-FAP and 296 subjects who remained asymptomatic. This analysis provides insight into the likely symptoms of disease onset in subjects converting from asymptomatic to symptomatic TTR-FAP. This insight is useful in optimizing disease diagnosis and treatment.
A pedigree analysis of the French family carrying Y78F TTR mutation

N Magy-Bertrand¹, J Hardwick², S Valleix¹, H Gil¹, MD Benson²

¹ Internal medicine department, University Hospital, Besançon, France. ² Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA. ³ Medical Genetic laboratory, Hôpital Necker-Enfants malades, Paris, France.

nadine.magy@univ-fcomte.fr

INTRODUCTION: In 2003 we described the first family presenting with Y78F TTR mutation. The clinical features of the propositus were an axonal polyneuropathy and carpal tunnel syndrome. The disease onset was late at about 60 years of age. In 2011, we reported the third affected family member, a woman presenting with elastorrhexia, macroGLOSSIA, skin lesions accompanied with enlarging salivary glands but without polyneuropathy. In 2011, eleven family members carried the mutation and 2 have the disease. We can now report an extensive pedigree analysis of the family.

MATERIAL AND METHODS: All family members were contacted and informed of the presence of the disease in the family. Most of them agreed to have a genetic testing for the Y78F mutation by automatic sequencing of TTR DNA.

RESULTS: In the second generation (figure 1) 9 of the 10 members were tested and all 9 of them were positive for Y78F mutation (heterozygosity). Five of those nine members have TTR amyloidosis. Six of the 10 members are now deceased. In two of the five affected members in the second generational level, a monoclonal gammopathy was identified. In the third generational level, 23/32 members were tested. Thirteen family members were positive for the Y78F mutation and one, the older one, had the disease (axonal polyneuropathy). Most of the affected members had an axonal polyneuropathy and carpal tunnel syndrome. The clinical presentation described in 2011 seems to be isolated.

DISCUSSION AND CONCLUSION: The penetrance of the mutation was elevated in the second generation and decreased in the third generation. The follow-up of the 12 members of the third generation is planned to detect the occurrence of the disease.


Fig.1: Genealogical tree of the French family carrying Y78F TTR mutation
Is deep breathing a useful test of cardiac autonomic function in hereditary transthyretin amyloidosis?

Urban Wiklund a, Amir Kadkhodaee a, Kennet Andersson a, Ole B Suhr b, Rolf Hörnsten c

a Department of Radiation Sciences, Biomedical Engineering, Umeå University, Umeå, Sweden.
b Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden.
c Department of Surgical and Perioperative Sciences, Clinical Physiology, Heart Centre, Umeå University, Umeå, Sweden.

Background: The heart rate response to paced deep breathing is a common test of autonomic function. This study aims to assess the validity of the deep breathing test in patients with hereditary transthyretin amyloidosis (ATTR), where autonomic dysregulation as well as subtle atrial arrhythmias are common findings.

Methods: Heart rate fluctuations and respiration signals were retrieved from 96 recordings in 62 adult ATTR patients, and from 113 healthy subjects while performing paced deep breathing during one minute (six breaths/min). Autonomic function was scored by the average heart rate difference (deep breathing index, DBI). Additionally, the regularity of the signals was assessed using Fourier series analysis, estimating the fraction of the power of the signal that was found at the breathing frequency.

Results: Forty-nine percent of the recordings in ATTR patients presented with age-adjusted DBI scores below normal limits, indicating autonomic dysfunction. Although the other 51% of ATTR patients presented DBI scores within normal limits, only 30% were still considered as normal responses when the regulation in the heart rate signal was taken into consideration. Fourier series analysis revealed that the remaining 21% presented with irregular heart rate responses that were not synchronized with respiration. Instead, their autonomic scores were strongly influenced by subtle multifocal atrial arrhythmias and cardiac conduction disturbances. Thus, their autonomic function could not be evaluated by the deep breathing test.

Conclusion: Reduced heart rate fluctuations are common in ATTR patients during deep breathing, reflecting cardiac autonomic dysfunction. However, in ATTR patients presenting with “normal” values of traditional scores, the heart rate responses are often of non-autonomic origin and have to be carefully scrutinized, preferably by utilising Fourier series analysis to ensure that the heart rate is synchronized with respiration as shown in this study.

Figure 1. ATTR patient with no marked heart rate fluctuations before deep breathing, but who presented with high DBI due to three episodes of second-degree atioventricular block during deep breathing.
Transthyretin-related hereditary amyloidosis in an Argentine family with TTR Tyr-114Cys mutation

MA Aguirre, EM Nucifora, M Rugiero, M Chaves, P Sorroche, MS Saez, DH Giunta, BR Boietti, ML Posadas-Martínez

Department of Medicine, Instituto Universitario Hospital Italiano de Buenos Aires, Argentina. Grupo de Estudio de Amiloidosis.

adel.a.aguirre@hospitalitaliano.org.ar

Background: Transthyretin-related hereditary amyloidosis is an autosomal dominant inherited disease caused for mutations in the transthyretin (TTR) gene. Corresponding to the various transthyretin gene mutations and a wide range of geographical distribution, this disorder presents diverse characteristics in genotype-phenotype correlation.

Familial Amyloid Polyneuropathy (FAP) is the more common clinical presentation and the Val30Met its more frequent mutation described.

Aim: We describe the clinical characteristics of an Argentinian family affected by transthyretin-related amyloidosis with TTR Tyr114Cys mutation.

Methods/ Patient: We present a 65-year-old female patient with a history of 3 years of recurrent episodes of perspiration and dizziness followed by nausea, vomiting and syncope. She also developed distal paresthesia and progressive autonomic dysfunction: orthostatic hypotension, decreased libido and alternating diarrhea and constipation. She lost weight and had distal dysesthesias and muscle atrophy developed. Hypoesthesia in stocking-glove distribution and areflexia was found on physical examination. She did not showed symptoms or signs of CNS involvement. Amyloid deposition was found in sural nerve biopsy. MRI showed no leptomeningal lesion, cardiac evaluation and ocular examination were unremarkable.

Her oldest sister died, she had a biopsy proven amyloid leptomeningitis and her 52-year-old brother has the similar clinical picture.

TTR gene sequencing showed the Tyr114Cys mutation. Her son has the same mutation with no clinical signs nor symptoms.

Conclusion: we present an argentine family with TTR amyloidosis with a Tyr114Cys mutation, which is extremely rare and the first reported in our country.

References:

THE SWEDISH LANDSCAPE OF HEREDITARY ATTR-AMYLOIDOSIS.

Ole B Suhr, Jonas Wixner, Björn Pilebro, Intissar Anan.

Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden.

Background. Northern Sweden is a well known clustering area for hereditary transthyretin (TTR) amyloid (ATTR) amyloidosis caused by the Val30Met mutation. However, several additional mutations have emerged, of which several have not been reported outside of Sweden. The aim of the presentation is to give an overview of the various mutations found in the Swedish population.

Methods. All mutations and their phenotypes are presented in Table 1. The clinical presentation is derived from clinical records, and the mutations were in all cases settled by TaqMan analysis (Val30Met mutation) or by whole gene sequencing of the TTR gene. A possible origin of the family and the mutation from outside of Sweden was searched for when constructing pedigrees for each family.

Results. The 14 mutations found in the Swedish population as of Jan 2016 are outlined in Table 1. For the majority of mutations an ancestry outside of Sweden could not be settled even for mutations such as the Tyr60Ala and Gly53Glu. However, the Phe33Leu and Ala97Ser patients clearly originated from outside of Sweden. The Tyr69His mutation was first described in the USA, but it later became clear that the US-family originated from Sweden.

Discussion and conclusion

In Sweden, a large number of TTR mutations have emerged during the years, of which the majority appears to be spontaneous mutations within the Swedish population. Expectedly, polyneuropathy and/or cardiomyopathy were noted in the majority of patients.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Phenotype</th>
<th>Origin of proband or family</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val30Met</td>
<td>Polyneuropathy, gastrointestinal disturbances, eye deposits, cardiomyopathy, kidney complications</td>
<td>Sweden</td>
</tr>
<tr>
<td>Val30Leu</td>
<td>Polyneuropathy, cardiomyopathy. Gastrointestinal disturbances</td>
<td>Finland</td>
</tr>
<tr>
<td>Phe33Leu</td>
<td>Polyneuropathy, cardiomyopathy. Gastrointestinal disturbances</td>
<td>Poland</td>
</tr>
<tr>
<td>Ala45Ser</td>
<td>Cardiomyopathy, carpal tunnel syndrome</td>
<td>Sweden</td>
</tr>
<tr>
<td>Ala45Asp</td>
<td>Gastrointestinal dysfunction, polyneuropathy, cardiomyopathy</td>
<td>Ethiopia</td>
</tr>
<tr>
<td>Gly53Glu</td>
<td>CNS-involvement, cardiomyopathy</td>
<td>Sweden</td>
</tr>
<tr>
<td>Leu55Gln</td>
<td>Polyneuropathy, cardiomyopathy, gastrointestinal disturbances</td>
<td>Serbia</td>
</tr>
<tr>
<td>Gly57Arg</td>
<td>Cardiomyopathy, polyneuropathy</td>
<td>Sweden</td>
</tr>
<tr>
<td>Tyr60Ala</td>
<td>Cardiomyopathy, polyneuropathy, gastrointestinal disturbances</td>
<td>Sweden</td>
</tr>
<tr>
<td>Tyr69His</td>
<td>CNS complications, eye.</td>
<td>Sweden</td>
</tr>
<tr>
<td>Glu89Lys</td>
<td>Polyneuropathy, cardiomyopathy</td>
<td>Sweden, Malaysia/China</td>
</tr>
<tr>
<td>His88Arg</td>
<td>Cardiomyopathy, carpal tunnel syndrome</td>
<td>Sweden</td>
</tr>
<tr>
<td>Ala97Ser</td>
<td>Polyneuropathy, gastrointestinal disturbances, eye deposits, cardiomyopathy</td>
<td>Malaysia/China</td>
</tr>
<tr>
<td>Val122Ile</td>
<td>Cardiomyopathy</td>
<td>USA</td>
</tr>
</tbody>
</table>
Transthyretin amyloidosis: towards a novel therapeutic strategy

L Saelices1, LM Johnson1, WY Liang1, MR. Sawaya1, D Cascio1, P Ruchala2, J Whitelegge2, L Jiang3, R Riek, DS Eisenberg1

1Department of Biological Chemistry, the Department of Chemistry and Biochemistry, Molecular Biology Institute, and Howard Hughes Medical Institute, University of California, Los Angeles, Los Angeles CA, USA. 2Department of Psychiatry and Biobehavioral Sciences, University of California at Los Angeles, Los Angeles, CA 90024, USA; The Pasarow Mass Spectrometry Laboratory, The Jane and Terry Semel Institute for Neuroscience and Human Behavior, Los Angeles, CA 90024, USA. 3Department of Neurology, University of California, Los Angeles, Los Angeles CA, USA.

lorenasaelices@mbi.ucla.edu

INTRODUCTION: Transthyretin (TTR) is a transporter of the thyroid hormone T4 and retinol binding protein in blood and cerebrospinal fluid. With aging or by genetic inheritance, TTR abnormally aggregates into unbranched long fibrils that accumulate in different organs which eventually fail causing the life-threatening transthyretin amyloidosis [1]. Currently, there is no cure for transthyretin amyloidosis, and the standard first-line treatment of familial TTR amyloidosis is the undesired liver transplantation, which is often not enough to stop disease progression. Recent efforts into stabilizing the tetrameric non-amyloidogenic form of TTR led to compounds such as tafamidis [2], approved in Europe for the treatment of familial amyloidotic polyneuropathy. Additional approaches are needed to prevent the development of transthyretin amyloidosis. In our previous work, we developed a novel strategy to block fibril formation by small non-natural peptides [3].

MATERIAL & METHODS: We combined mutational and crystallographic structural studies of TTR variants and isolated peptides to uncover the involvement of the strands F and H in TTR aggregation. Based on the amyloid crystal structures of strands F and H, we designed non-natural peptide inhibitors which were recently optimized by Rosetta energy prediction. We followed aggregation of recombinant TTR by (i) measuring absorbance of the sample at 400 nm, (ii) measuring the protein concentration of the insoluble fraction and (iii) electron microscopy. Additionally, the amyloid fibrils extracted from cardiac tissue of a transthyretin amyloidosis patient was used to seed the aggregation of recombinant wild-type and mutant TTR. Furthermore, we used an amyloidosis model of Drosophila melanogaster to analyze the effectiveness of the inhibitors in vivo. To do so, adult flies were fed inhibitors and behavioral phenotype and motor skills were analyzed in climbing assays. The inhibitors were evaluated both in the absence and the presence of tetramer stabilizers.

RESULTS: The blockers not only hindered aggregation of the protein in vitro [3] but also stopped generation of new fibrils formed from extracted patient tissue. Moreover, its mechanism of action allowed combinatory treatment with tetramer-stabilizing compounds. Preliminary results in flies showed a significant improvement of motor skills when treated with the inhibitors, which corresponded to an increase of the soluble fraction of transthyretin in fly homogenates.

DISCUSSION & CONCLUSIONS: This work provides the first characterization of peptide inhibitors for TTR aggregation, establishing a novel therapeutic strategy for transthyretin amyloidosis.

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Impaired left ventricular compliance in cardiac amyloidosis – the unraveling role of leg lifting test

B Pilebro1, MY Henein1, S Arvidsson1, C Backman2, OB Suhr1, P Lindqvist3

1 Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden. 2 Division of Clinical Physiology, Heart Centre, Umeå University Hospital, Umeå, Sweden. 3 Department of Surgical and Perioperative Sciences, Umeå University, Umeå, Sweden.

Introduction: Apart from increased wall thickness of > 12 mm, the nature of left ventricular (LV) involvement in cardiac amyloidosis due to hereditary transthyretin (cATTR) is not well defined. Patients might present with only mildly increased wall thickness but also with symptoms despite no signs of significantly raised LV filling pressures.

Material & Methods: The aim of this study was to assess the response of LV Doppler echocardiographic filling indexes to acute modulation of preload by 2 minutes leg lifting in a group of 20 cATTR patients (age 69±8 years, 20 males, 60% had fragmented fibril type) and compare them with 35 healthy controls (age 59±12 years, 16 males). 2D strain was also used to assess longitudinal LV systolic deformation (GLS), LV compliance by measuring early diastolic strain rate (LVSRe) as well as left atrial (LA) deformation during ventricular (PALS) and atrial systole (LASRa).

Results: Patients were older and had higher body surface area, and conventional measurements of LV dimensions and diastolic function (E, A and E/A) at rest were not different from controls, however LV wall thickness, LA volume and E/e’ was higher and myocardial deformation as well as LVEF were lower (p<0.01). With leg lifting, E velocity increased in both groups but LVSRe increased only in controls and was attenuated in patients, suggesting reduced LV compliance during preload increase. This was further supported by the additional fall in LASRa and a higher E/e’ (p<0.001) in cATTR, table 1. Also, LV wall thickness correlated with leg lifting E/e’ (r=0.61, p<0.001), LVSRe (r=-0.49, p<0.001) and LASRa (r=0.35, p<0.05).

Discussion & Conclusions: Despite no difference in E/A flow pattern at rest, patients with cATTR exhibit severe diastolic dysfunction and clear signs of poor myocardial compliance during preload test. These signs can easily be unraveled with simple leg lifting test.

Table 1. Clinical and echocardiographic findings in patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (35)</th>
<th>cATTR (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td>59±12</td>
<td>69±8*</td>
</tr>
<tr>
<td><strong>Rest</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV, ml</td>
<td>77±15</td>
<td>79±22</td>
</tr>
<tr>
<td>LA volume, ml</td>
<td>41±13</td>
<td>77±28†</td>
</tr>
<tr>
<td>LVEE, %</td>
<td>60±6</td>
<td>51±10†</td>
</tr>
<tr>
<td>IVSD, mm</td>
<td>9.6±1.6</td>
<td>19.2±3.5†</td>
</tr>
<tr>
<td>E/A</td>
<td>1.1±0.3</td>
<td>1.4±0.8</td>
</tr>
<tr>
<td>LV GLS, %</td>
<td>-19±3</td>
<td>-13±5†</td>
</tr>
<tr>
<td>PALS, %</td>
<td>28±9</td>
<td>13±9†</td>
</tr>
<tr>
<td>LV GLE, SR</td>
<td>1.1±0.3</td>
<td>0.8±0.4†</td>
</tr>
<tr>
<td>E/e’</td>
<td>8.5±2.4</td>
<td>11.9±4.2†</td>
</tr>
<tr>
<td>LASRa, 1/s</td>
<td>-1.5±1.1</td>
<td>-1.2±0.8</td>
</tr>
<tr>
<td><strong>Passive leg lifting</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SV, ml</td>
<td>82±18</td>
<td>81±21</td>
</tr>
<tr>
<td>LV GLS, %</td>
<td>-19±3</td>
<td>-11±8†</td>
</tr>
<tr>
<td>PALS, %</td>
<td>28±9</td>
<td>15±12‡</td>
</tr>
<tr>
<td>LASRa, 1/s</td>
<td>-1.4±0.8</td>
<td>-0.8±1.1‡</td>
</tr>
<tr>
<td>E/e’</td>
<td>7.9±2.5</td>
<td>11.9±4.8‡</td>
</tr>
<tr>
<td>LV GLE SR</td>
<td>1.4±0.4</td>
<td>0.8±0.3‡</td>
</tr>
</tbody>
</table>

* p<0.05, † p<0.001 from controls
Reduction of Both Wild-Type and Mutant TTR Across Nine Different Mutations in FAP Patients Treated with IONIS-TTRRx Using a Mass Spectrometric Immunoassay

PJ Nelson1, MD Benson1, BP Monia2, H Gaus2, S Guo2, EJ Ackermann2

1 Indiana University School of Medicine, Department of Pathology and Laboratory Medicine, Indianapolis, IN USA. 2Ionis Pharmaceuticals

INTRODUCTION: Misfolding of the plasma protein transthyretin (TTR) and its deposition in the body’s organs and tissues causes Transthyretin Amyloidosis (ATTR). Patients with the hereditary form of the disease (familial amyloid polyneuropathy or familial amyloid cardiomyopathy) are typically heterozygotes, harbouring one wild-type (wt) allele and one allele coding for one of more than 100 possible disease-causing TTR mutations. Even though mutant TTR appears to drive the amyloid deposition process, it has been well documented that amyloid deposits in these patients contain both wt and mutant TTR, and after liver transplantation, wt TTR may continue to deposit on existing amyloid fibrils. One therapeutic strategy under clinical development is to reduce the production of all forms of TTR protein produced by the liver by utilizing a Gen 2+ antisense oligonucleotide (IONIS-TTRRx). IONIS-TTRRx is designed to bind to a region on the 3’UTR of the TTR mRNA which is conserved on all TTR mRNA transcripts. This approach may enable the therapeutic use of IONIS-TTRRx in all hereditary ATTR patients regardless of the specific TTR mutation, as well as in patients with the non-hereditary form of the disease caused exclusively by wt TTR. Recently a LC-MS assay was developed to independently quantify the levels of wt and mutant TTR protein in human serum. Utilizing this assay we examined the baseline and post-treatment levels of wt and mutant TTR protein from nine patients harbouring distinct TTR mutations who participated in the NEURO-TTR Phase 3 study and its open-label extension (OLE) study.

MATERIAL & METHODS: Wt and mutant TTR protein was extracted from human serum of FAP patients by Thermo TTR mass spectrometry immunoassay (MSIA®) Disposable Automation Research Tips (D.A.R.T’s®). His-tagged recombinant human transthyretin was pre-spiked as the internal standard. Proteins were then eluted from MSIA tips, reduced by TCEP-HCl and followed by chromatographic separation on a monolithic column. Accurate and high resolution mass detection of intact TTR proteins were performed by a Thermo Q-Exactive Hybrid Quadrupole-Orbitrap mass spectrometer in full scan mode. Due to the lack of pure analytes, calibration curves were generated by spiking recombinant human TTR standard in normal human serum at known concentrations, followed by extraction and mass analysis using the same procedures. Relative concentrations of TTR protein in patients’ serum were determined using peak area ratios of TTR to His-TTR. The identification of wt or mutant TTR proteins was achieved by protein deconvolution.

RESULTS: Analysis of the baseline levels of wt and mutant TTR from nine patients each with a different TTR mutation (Val30Met, Ser77Phe, Asp38Ala, Tyr114Cys, Ile84Ser, Phe64Leu, Ile107Val, Leu58His, and Thr60Ala) showed that the LC-MS assay could identify and quantitate the levels of wt-TTR and mutant TTR for all nine TTR mutations tested. Results showed a TTR mutant/wt protein ratio at baseline ranging from 0.7-1.0 consistent with previous reports. Analysis of post-treatment TTR levels during the OLE study showed robust and approximately equal reduction in the levels of both wt and mutant TTR. These results correlated strongly with results obtained with the commonly used immunoturbometric assay which quantifies total TTR (wt + mutant).

DISCUSSION & CONCLUSIONS: IONIS-TTRRx effectively reduces the levels of both wild-type and mutant TTR across multiple mutations in patients with FAP.
PB33
A new and unusual transthyretin mutant, duplication of TTR codons 51 and 52 (Glu51_Ser52dup), associated with severely aggressive familial amyloidotic polyneuropathy
B Spencer, JL Berk, E Klimtchuk, P Soo Hoo, T Prokaeva, LH Connors
Amyloidosis Center, Boston University School of Medicine, Boston, Massachusetts, USA.
bspencer@bu.edu

INTRODUCTION: Transthyretin amyloidosis (ATTRm) is the most common form of familial amyloidosis. Thus far, more than 100 amyloidogenic TTR mutations\(^1\) have been identified throughout all 4 coding regions of the gene. Usually, the amyloid fibril-forming mutant proteins contain single amino acid substitutions. We now present a new and unusual mutation characterized by codon duplication in exon 3 of the TTR gene and featured in a highly aggressive phenotype of ATTRm amyloidosis.

MATERIAL & METHODS: A diagnosis of amyloidosis was established by Congo red staining of a tissue biopsy; identification of TTR as the amyloid deposited protein was accomplished by mass spectral analysis. Isoelectric focusing was used to screen serum for a TTR variant protein. Mutation identification was accomplished by direct nucleotide sequencing of exons 1-4 in the TTR gene using DNA extracted from blood leukocytes.

RESULTS: A 38 year old, African-American male presented with symptoms of sensorimotor peripheral polyneuropathy and mild myopathy. At first visit, the patient reported experiencing symmetrical numbness with pain below the knees, pain in his hands with no numbness or tingling, and mild leg weakness over the course of the last twelve months. A review of the patient’s family history showed that the father had been diagnosed with familial amyloidosis featuring heart failure and peripheral neuropathy, and had died at age 46. Other family members reporting “foot problems” possibly related to amyloidosis included 4 uncles and 2 aunts. One brother, with sural nerve biopsy proof of amyloid, died at age 34; symptoms of peripheral and autonomic neuropathy with possible cardiac involvement were reported in this sibling.

Three months prior to evaluation, the patient was diagnosed with amyloidosis by Congo red staining of right shin skin biopsy; mass spectral analysis of the congophilic deposits identified TTR as the amyloid fibril protein. At evaluation, clinical and laboratory findings included: intact motor function except for 4/5 dorsiflexion of the big toe with normal plantar function and decreased sensation from the feet to below the knees consistent with sensorimotor polyneuropathy of lower extremities; myopathy in the legs with elevated creatinine kinase at 563 U/L, mildly increased left ventricular wall thickness of 13 mm, and mild diastolic dysfunction with negative cardiac biomarkers consistent with no evidence of cardiac amyloidosis.

Congo red staining of a fat pad aspirate was strongly positive (2-3+) for congophilic deposits. Serum screening by IEF indicated that wild-type and variant forms of TTR were present. DNA sequencing of TTR exons 1-4 showed heterozygosity for a two codon duplication (c.151_156dupGAGTCT) in exon 3 corresponding to a two amino acid residue insertion (p.Glu51_Ser52dup); no other abnormalities were detected.

The patient was started on diflunisal, a non-steroidal anti-inflammatory drug recently shown to have efficacy in ATTRm\(^2\). However, in this patient, peripheral polyneuropathy and myopathy were unabated and disease progression included development of autonomic neuropathy. After 19 months, treatment with diflunisal was halted. In less than 4 years from initial evaluation, the patient died of pneumonia and heart failure.

DISCUSSION & CONCLUSIONS: This is the first report of codon duplication in the TTR gene which causes a severely aggressive form of ATTRm amyloidosis. The unusual and pathologic genotype features expression of variant protein with a two amino acid insertion and a phenotype characterized by rapidly progressing peripheral and autonomic neuropathies, and myopathy.


This research was supported by the Boston University Amyloid Research Fund, the National Institutes of Health RO1AG031804 (LHC), and the Young Family Amyloid Research Fund.
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The demographic, genetic, and clinical characteristics of Brazilian subjects enrolled in the Transthyretin Amyloidosis Outcomes Survey

M Waddington Cruz¹, D Foguel¹, AC Berensztejn¹, RC Pedrosa¹, R Mundayat², M-L Ong², on behalf of THAOS Investigators

¹ Federal University of Rio de Janeiro, National Amyloidosis Referral Center, CEPARM, Rio de Janeiro, Brazil. ² Pfizer Inc, New York, NY, USA.

mwaddingtoncruz@gmail.com

INTRODUCTION: Hereditary transthyretin (TTR) amyloidosis is a rare, fatal disorder resulting from mutated TTR genes leading to amyloid accumulation in nervous tissue and various organs. Portugal and Brazil, a country of Portuguese colonization, are considered endemic regions, along with Sweden and Japan. Although a common haplotype was observed between Portuguese and Brazilian patients with TTR amyloidosis, much of the Brazilian population is racially heterogeneous with native Indian and African influences. The objective of the present work was to characterize the demographic and clinical characteristics of Brazilian subjects with TTR amyloidosis given their Portuguese origin, yet racial heterogeneity.

MATERIAL & METHODS: Baseline demographic and clinical characteristics were obtained from Brazilian subjects enrolled in the ongoing, international Transthyretin Amyloidosis Outcomes Survey (THAOS). The analysis (cut-off date: January 14, 2016) was performed on subject data collected during clinical evaluations and from a variety of standard assessments. The outcome measures included demographics (age of symptom onset, gender, time from onset of symptoms to diagnosis, family history), genotype, and clinical characteristics (presence of amyloid deposit, frequency of misdiagnosis, presenting symptomatology).

RESULTS: In total, 160 subjects (52.5% male) were included in the analysis, comprising those with Val30Met (n=147, 91.9%) and non-Val30Met (n=13, 8.1%) mutations and a median age at symptom onset of 32.5 years. Median time from symptom onset to diagnosis for men and women was 2.6 years and 5.0 years, respectively. In total, 109 (68.1%) subjects were Caucasian, 22 (13.8%) were Latino American, 1 (0.6%) was of African descent, and 28 (17.5%) were listed as other. The majority of subjects (90.6%) reported a known family history of symptomatic TTR amyloidosis. Amyloid deposit was found in 80.8% of the biopsies performed in symptomatic subjects (the most common tissue type tested was salivary gland followed by nerve). Misdiagnosis was observed in 26.6% of the symptomatic cases (26.1% of Val30Met mutations and 33.3% of non-Val30Met mutations), with chronic inflammatory demyelinating polyneuropathy (CIDP) being the most common diagnosis. Over one-third (35.3%) of the patients experienced a delay of more than 1 year to receive a correct diagnosis. Of the 128 symptomatic patients at enrollment, 79.7% presented with motor neuropathy (80.7 % Val30Met and 66.7% non-Val30Met); 87.5% presented with sensory neuropathy (88.2% Val30Met and 77.8% non-Val30Met); 93.8% presented with autonomic neuropathy (94.1% Val30Met and 88.9% non-Val30Met); and 82.8% presented with gastrointestinal complaints (84.0% Val30Met and 66.7% non-Val30Met). Unintentional weight loss was noted in 50.8% of the cases. When considering age of onset, 82.0% of early onset cases and 74.1% of late onset cases presented with motor neuropathy. Corresponding values for sensory neuropathy were 90.0% (early onset) and 81.5% (late onset), and for autonomic neuropathy were 95.0% (early onset) and 88.9% (late onset). Cardiac disorders were present in 35.2% of symptomatic subjects (32.8% Val30Met and 66.7% non-Val30Met).

DISCUSSION & CONCLUSIONS: The Brazilian population of subjects enrolled in THAOS was primarily characterized by early onset TTR amyloidosis with a Val30Met mutation and neuropathic phenotype; several cases also involved cardiac disease. This profile is highly similar to those from endemic regions of Portugal, despite several generations of Portuguese descent and a racially heterogeneous population.
Characterization of sera from FAP patients by cellular toxicity assessments

C Niemietz, G Chandhok, V Sauer, S Guttmann, Y Avsar, A Zibert, HH Schmidt

Klinik für Transplantationsmedizin, Universitätsklinikum Münster, Münster, Germany.
hepar@ukmuenster.de

INTRODUCTION: Phenotypic presentation of familial amyloid polyneuropathy (FAP) is diverse and a considerable variation in symptoms is noticed among individuals and across geographic regions. Diagnosis is mostly based on the presence of peripheral or autonomic neuropathy, in addition to one or more other clinical findings, including a family history of neuropathy, cardiac hypertrophy, renal and/or ocular involvement. Typically, amyloid deposits are observed in biopsies of FAP patients. Due to the variety of the clinical findings and the rare incidence of the disease, many patients can only be diagnosed several years after onset of the disease. Besides genetic testing of the transthyretin (TTR) gene there is no single biochemical assay to confirm FAP diagnosis. In vitro, purified TTR derived from E.coli was reported to be toxic when incubated with tissue culture cells. We addressed the question whether sera of FAP patients might be toxic to cells.

MATERIAL & METHODS: The neuronal cell line IMR-32 was seeded in triplicates in a 96 well plate and cultivated overnight in 100 µl DMEM medium lacking phenol red. Sera of FAP patients were added thereafter to the medium. After 7 days of incubation, the cell viability was determined by an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay and the respective absorbance was determined. For Tafamidis or antibody testing, sera were preincubated for at least 2 h. Results were calculated as a percentage of the untreated cells.

RESULTS: Sera of FAP patients induced a high amount of toxicity in IMR-32 cells (74.5 ± 34%). In contrast, in sera from healthy individuals a low toxicity was observed (15.7 ± 10%). Preincubation of FAP sera with Tafamidis could significantly reduce toxicity by 21.2 ± 16% in FAP sera, whereas no effect of Tafamidis preincubation was observed in sera of healthy individuals (3.8 ± 8%). In addition, preincubation of FAP sera with polyclonal antibody directed against human TTR could significantly reduce toxicity.

DISCUSSION & CONCLUSIONS: Our preliminary data indicate that sera of FAP patients exhibit toxicity that is related to mutated TTR as suggested by Tafamidis and antibody preincubation experiments. The assessment of toxicity observed in sera of FAP patients could be valuable to support the tedious diagnostic evaluation of patients as well as to monitor efficacy of treatment.
PB36
Serum proteomic variability associated with clinical phenotype in familial transthyretin amyloidosis (ATTRm)
LH Connors1,2, CM Koch1,2, GG Chan1
1Gerry Amyloidosis Research Laboratory in the Amyloidosis Center and 2Department of Pathology and Laboratory Medicine, Boston University School of Medicine, Boston, Massachusetts, USA
lconnors@bu.edu

INTRODUCTION: Transthyretin (TTR), normally a plasma circulating protein, can become misfolded and insoluble, forming extracellular deposits of amyloid fibrils usually targeted to heart or nerve tissues. This group of diseases, referred to as ATTR amyloidosis, is frequently life-threatening and fatal if untreated. ATTR amyloidosis caused by mutant proteins (ATTRm), which arise from point mutations in the TTR gene, were classically referred to as familial amyloid polyneuropathy (FAP) or familial amyloid cardiomyopathy (FAC) reflecting the clinical phenotype. FAP and FAC are rare diseases that are frequently difficult to diagnose and biomarkers of amyloidosis onset and progression are yet to be identified. Thus, the discovery of disease-specific indicators has the potential to improve diagnosis, enable accurate measurement of disease course and response to treatment, and reveal key information regarding disease mechanisms. This study was undertaken to investigate serum proteomic features unique to FAP and FAC.

MATERIAL & METHODS: Sera from age-, gender-, and race-matched cases of ATTR-FAP, ATTR-FAC, and controls were selected for this study. Experimental samples were prepared by pooling equivalent amounts of serum from 10 cases in each group. Analyses of patient and control sera were performed using PeptiQuant™ Human Discovery Assay (MRM Proteomics, Inc.) to determine the presence and relative concentrations of 192 human proteins in the samples. The ATTR-FAP group included ATTR-V30M, -L58H, and -K70N; in the ATTR-FAC group, cases of ATTR-A19D, -T59K, -T60A, -I68L, -A81V, and -E89Q were represented. Patient and control groups were compared using the Welch t-test for unequal variance and data was adjusted with the false discovery rate (FDR) test; p < 0.05 indicated statistical significance. Proteomic data unique to ATTR-FAP or ATTR-FAC were analyzed using DAVID to identify functional annotations and STRING to predict protein-to-protein interactions.

RESULTS: Comparisons of patient and control groups showed significant differences in serum levels of 107/192 (56%) proteins. In ATTR-FAP, 12 proteins were found to be unique to that group; 15 proteins, including TTR, were distinct to ATTR-FAC (Figure 1A, B). In the disease-specific protein sets, serum concentrations were generally lower than controls and DAVID identified signal, signal peptide, and extracellular region (Figure 1A, B). Predicted interactions using STRING failed to identify any reported associations among the ATTR-FAP protein group (Figure 1C); however, in ATTR-FAC-specific proteins, interactions were noted and classified as activation, binding, catalysis, and reaction (Figure 1D).

DISCUSSION & CONCLUSIONS: Our results show significant differences in the serum proteomes of patients with ATTR-FAP and ATTR-FAC, and age-, gender-, and race-matched healthy controls. Protein concentrations, interactions, and functions unique to ATTR-FAP and ATTR-FAC were identified. Interestingly, TTR was unique to ATTR-FAC, but not ATTR-FAP. These data provide evidence that disease-specific protein differences in ATTR-FAP and ATTR-FAC do occur and may have diagnostic and prognostic utility. This research was supported by the E. Rhodes and Leona B. Carpenter Foundation Grant, National Institutes of Health RO1AG031804, and the Young Family Amyloid Research Fund.

Figure 1. Heat map showing DAVID functional terms relating to proteins unique to (A) ATTR-FAP or (B) ATTR-FAC; GREEN = reported annotated terms, BLACK = unreported annotated terms. STRING predicted associations among unique protein sets in (C) ATTR-FAP and (D) ATTR-FAC.
PB37

HEREDITARY TRANSTHYRETIN AMYLOIDOSIS ASSOCIATED WITH A TRANSTHYRETIN VARIANT THR59ARG

Tetsuya Watanabe1,4, Konen Obayashi2, Yohei Misumi1, Masayoshi Tasaki2, Satoru Shinriki3, Tomotaka Ando6, Takafumi Akagami7, Mitsuharu Ueda1, Taro Yamashita1,5, Shinichi Hirotani6, Yukio Ando1

1 Department of Neurology, 2 Department of Morphological and Physiological Sciences, 3 Department of Laboratory Medicine, Graduate School of Medical Sciences, 4 Department of Molecular Physiology, Faculty of life Sciences, Kumamoto University, 5 Diagnostic Unit for Amyloidosis, Department of Neurology, Kumamoto University Hospital, Kumamoto, Japan.

6 Cardiovascular Division/Division of Coronary Heart Disease, 7 Division of Diabetes and Metabolism, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan.

tetsuya.w09@gmail.com

INTRODUCTION: Hereditary transthyretin (TTR) amyloidosis is characterized by TTR amyloid deposits in various tissue sites and organs, such as peripheral nerves, heart, gastrointestinal tract, kidneys, eyes, and leptomeningeal tissues of the brain. So far, more than 130 mutations in the TTR gene have been found in the worldwide and genotype-phenotype correlations were reported in hereditary TTR amyloidosis patients. Here we report a Japanese patient having TTR Thr59Arg mutation with severe cardiac symptoms.

CASE REPORT: A 52-year-old Japanese man had exercise-induced dyspnea and pitting edema in his legs. Cardiac arrhythmias and left anterior fasciculator block and left ventricular wall thickness were detected. He also showed mild peripheral autonomic neuropathy. Other typical FAP manifestations were not observed. His family history of FAP was not revealed. Massive TTR amyloid deposits were detected in histopathological analyses. DNA sequence analysis of the TTR gene revealed C-to-G transition in codon 59 of exon 3, which cause a mutation of TTR Thr59Arg. Using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry, both wild-type TTR and TTR Thr59Arg variant peaks were also detected.

CONCLUSION: A TTR Thr59Arg mutation may cause severe amyloid cardiomyopathy and mild amyloid polyneuropathy.

REFERENCE:

PB38
A case with a novel variant transthyretin A36D presenting cardiac phenotype

T Nomura, T Yamashita, Y Misumi, M Ueda, T Masuda, M Tasaki, A Fujimoto, T Amano, Y Ando

Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan
caramelbox0106@gmail.com

INTRODUCTION
As of today, more than 130 point mutations and a deletion in the transthyretin (TTR) gene have been reported as a cause of hereditary TTR amyloidosis, and several phenotypes of FAP, including the polyneuropathic, oculoleptomeningeal, and cardiac types, have been reported. We have identified a novel mutation in the TTR gene substituting aspartic acid for alanine at position 36 with systemic amyloidosis in a Japanese woman in whom cardiac involvement was the major feature.

METHODS
We investigated clinicopathological characteristics of a case with hereditary TTR amyloidosis caused by a novel mutation: TTR A36D (p.A56D).

RESULTS
The case was a 59-year-old Japanese woman who had a 2-year history of progressing dyspnea on exertion. Echocardiography showed concentric ventricular hypertrophy, atrial dilatation, and dystelectasis with a granular sparkling pattern. Myocardial biopsy revealed severe TTR amyloid deposition demonstrated by Congo red staining, immunohistochemistry, and LC-MS/MS analyses. DNA sequence analysis confirmed a novel ATTR A36D (p.A56D) mutation. She also presented with mild sensory neuropathy, however, muscle weakness, autonomic dysfunction, and eye manifestation were not observed. She was treated with tafamidis, a TTR stabilizer, and cardiac symptoms remained unchanged for 9 months.

DISCUSSION & CONCLUSION
We have reported a clinical feature of a patient with systemic amyloidosis caused by a novel TTR mutation (ATTR A36D) presenting dominant cardiac phenotype. The effects of tafamidis, liver transplantation, and gene silencing therapy should be evaluated as possible therapeutic approaches for this mutation.
PB39

Doxycycline-Tauroursodeoxycholic acid treatment: effects in the heart of a Transthyretin V30M transgenic mouse model

CA Teixeira, S Costelha, MJ Saraiva

Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Portugal;

Unidade de Neurobiologia Molecular, IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal

cristina.teixeira@ibmc.up.pt

Introduction: Until fairly recently, the number of people affected by cardiac amyloidoses was dramatically underrated mostly due to misdiagnosis. Not only this is the most fatal manifestation of systemic amyloidoses [1], it is now recognized that transthyretin (TTR) amyloidosis is relatively common affecting millions of people worldwide. Our research work is currently focused in the characterization of TTR V30M involvement in heart function through (i) the analysis of physiologic and/or cellular and biochemical parameters in \textit{in vitro} and \textit{in vivo} models; ii) study alternative therapeutic candidates aiming to enhance deposit clearance, ameliorate heart function, and counteract toxicity-related pathways; iii) and, ultimately to establish new biomarkers for clinical application.

Material and Methods: For this work we used 22-months old hTTR V30M transgenic mice deficient for the heat shock factor-1. The animals were treated with both Doxy (IP injection) TUDCA (in the drinking water) for four weeks while an age-matched control group was treated with vehicles (PBS and regular tap water respectively). After animal sacrifice, we analysed and compared the amyloid deposition in the GI tract by Congo-Red staining, TTR deposition in the heart by immunohistochemistry, collagen IV mRNA expression by qRT-PCR and B-type natriuretic peptide (BNP) levels in the serum by ELISA. Previous results from our lab showed the ability of Doxycycline/TUDCA treatment in reducing TTR deposition in the GI tract of V30M mice [2].

Results: More than 50% of the untreated animals presented TTR amyloid in the GI tract whereas only 9% of the treatment group exhibited amyloid deposition. TTR deposition in the heart occurred mostly in the extracellular matrix (ECM) and in cells other than cardiomyocytes. We observed a decrease in mRNA levels of collagen IV by qRT-PCR in the treated group as compared with the control group. The treatment also led to a decrease in serum levels of BNP.

Discussion and Conclusions: These results provide a starting point in the characterization of this mouse model regarding heart function and its relationship with TTR burden. Further studies will be conducted regarding signalling cascades and alternative drugs for their effect in preventing toxicity and improving heart function in animals affected by TTR deposition.

References:

Development of a care manual for familial amyloid polyneuropathy

C Kukinaka¹, H Kawasaki², S Nakagomi³, H Kokufu¹, T Yamashita⁴, Y Ando⁴

¹ Department of Nursing Faculty of Life Science Kumamoto University. ² Graduate School of Health Science, Hiroshima University. ³ University of Yamanashi, Graduate School of Medical Science. ⁴ Department of Diagnostic Medicine, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan.

kukinaka@kumamoto-u.ac.jp

INTRODUCTION

In previous studies, it was discovered that Japanese nurses find it difficult to deal with patients with familial amyloid polyneuropathy (FAP) and their families in various aspects. This disease is inherited in an autosomal dominant manner. Also, it is very difficult to seek control disease compared with FAP. Since nursing for patients with genetic diseases tend to be strongly influenced by culture, ways of living which need to take into consideration. The aim of this study was to develop a care manual that enables Japanese nurses to care for FAP patients and their families to give them better life.

METHODS

The contents of the care manual were created in the following procedures.

1. Genetic nursing competency was selected after reviewing the literatures.

2. The contents of the care manual were decided based on the focus group interviews by nurses. The issues were also checked against the genetic nursing competency.

RESULTS

1. In the genetic nursing competency, the following aspects were found to be imperative: understanding of genetic diseases, understanding of genetic testing, and understanding how to write a genealogy. Moreover, understanding clients’ subjective experiences was shown to be important.

2. The following capabilities were necessary for Japanese FAP nurses: a skill to understand experience of FAP patients and their families, a skill to care for the complex symptoms, a skill to care in conjunction with a variety of occupations such as medical social workers and certified genetic counselors, and a skill to obtain the knowledge of clinical genetics.

3. A skill to understand the genetic problems faced by patients and their families was most important.

4. A flow chart and checklist for FAP treatment and care were created in order to facilitate the care and to support the decision making of patients’ welfare.

DISCUSSION & CONCLUSIONS

Since FAP is a rare disease as compared to cancer and diabetes, common nurses rarely encounter with FAP patients. Therefore, it is imperative to enable nurses to care for FAP patients by referring to the care manual and by utilizing a flowchart and checklist. We have created the care manual with a flowchart and checklist for FAP treatments, care, and decision making for the patients’ welfare. Further consideration is required in order to improve the quality of care for FAP.
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Two brothers homozygous for ATTR V30M both presenting with phenotypes dominated by central nervous complications

Y Uchida1,3, M Ueda2, T Yamashita2, Y Ando3, S Kobayashi3, K Takada1, Y Tsugu3, T Toyoda1, G Yamada1, N Matsukawa1

1Department of Neurology, Nagoya City University, Aichi, Japan. 2Department of Neurology, Kumamoto University, Kumamoto, Japan. 3Department of Neurology, Toyokawa City Hospital, Aichi, Japan.

shoryuken@hotmail.co.jp

INTRODUCTION: Central nervous system (CNS) amyloid deposition in patients with the amyloidogenic transthyretin (ATTR) V30M mutation has been thought to occur only in patients with longer disease duration, a feature that is a major factor in the emergence of this rare clinical phenotype [1].

MATERIAL & METHODS: We investigated the clinical, radiological, and pathological findings of two Japanese brothers (patients 1 and 2) both suffering from severe CNS symptoms. Brain biopsy specimens were used for histopathological investigations. To screen for TTR in serum, mass spectrometry analysis was used [2]. Diagnoses were made via genetic methods.

RESULTS: They had severe CNS symptoms induced by cerebral amyloid angiopathy (CAA) and leptomeningeal amyloidosis. Their magnetic resonance images manifested cortical superficial siderosis and diffuse leptomeningeal enhancement. Both patients were homozygous for a valine-to-methionine substitution at TTR gene position 30.

DISCUSSION & CONCLUSIONS: The present cases are to the best of our knowledge, the first reports of these complications in non-liver transplanted ATTR V30M patients. These illustrate the diversity of symptoms encountered in homozygous ATTR V30M patients.


Fig. 1: Magnetic resonance imaging (MRI) of patients 1 and 2 (A and B). The T1-weighted images after contrast agent administration during cranial MRI of patient 1 showed diffuse leptomeningeal enhancement along the Sylvian fissures (arrows) (A). Susceptibility-weighted imaging of patient 2 showed diffuse, low-signal-intensity symmetrical rims of the Sylvian and other fissures in the cortex (arrows) (B). Brain biopsy findings of patient 1 (C–E). Inspection revealed cotton-like, white materials in the subarachnoid space after a dural incision (C). Histological evaluation via Congo red staining (D) and polarized light analysis (E) revealed massive amyloid deposits in the cerebral leptomeninges, which included the white materials at the surface. Those amyloid deposits reacted with an anti-TTR antibody (DAKO, Glostrup, Denmark) (F). Scale bars=200 mm.
TRANSTHYRETIN VARIANTS IN A GERMAN COHORT WITH HEREDITARY AMYLOIDOSIS

Y Avsar1, C Roecken2, A Barreiros3, A Zibert4, H Schmidt4

1 Department of Transplant Medicine, University Hospital Muenster, Muenster, Germany.
2 Department of Pathology, Christian-Albrechts-University Kiel, Kiel, Germany.
3 Department of Neurology, University Hospital Regensburg, Regensburg, Germany.
4 avsar.ysm@gmail.com

INTRODUCTION: Hereditary amyloidosis is most commonly associated with a mutation of the transthyretin gene (TTR). With more than 100 amyloidogenic TTR-variants known so far, a widely variable phenotype is being observed. High frequency mutations have been detected in Western Europe, USA and Japan. Common mutations in Germany have yet to be identified.

MATERIAL & METHODS: The mutational pattern of TTR-amyloidosis in Germany was studied. 106 patients with histologically and genetically confirmed TTR-amyloidosis from 65 unrelated families were included. Patients were examined for clinical signs and symptoms.

RESULTS: The most prevalent mutation detected was Val30Met, identified in 52 individuals, including 26 patients from 9 families and 26 unrelated patients. Although all patients with this mutation presented primarily with polyneuropathy, a significant variation of age of onset was seen, ranging from 20 to 60 years. Further mutations identified in the remaining patients were Gly47Ala, Gly47Glu, Leu58His, Ile107Val, Gly53Ala, Asp39Val, Arg34Thr, Val20Ile, Glu89Gln, Thr59Lys, Thr60Ala, Ile107Met, Glu54Gln, Tyr114Cys, Thr96Asn, Asp38Val and Val122Ile. 16/106 patients received a liver transplant and 21/106 patients are currently being treated with Tafamidis.

DISCUSSION & CONCLUSIONS: This is the first study of the mutational pattern of patients with TTR-amyloidosis in Germany. Similar to the largest foci of hereditary amyloidosis in Portugal, Sweden and Japan, Val30Met was found to be the most common mutation, followed by Gly47Ala.


Fig. 1: Found mutations and their frequencies
Mitochondrial DNA copy number and new insights on biological mechanisms of Familial Amyloid Polyneuropathy

D Santos¹,², MJ Santos⁴,⁵, T Coelho³, M Alves-Ferreira¹,², J Sequeiros¹,², I Alonso¹,², A Sousa¹,², M Graziana*⁴,⁵, C Lemos*¹,²

*these authors contributed equally for supervision of this work

¹ UnIGENe, Instituto de Investigação e Inovação em Saúde (i3S) and Institute for Molecular and Cell Biology (IBMC), Univ. Porto; ² Instituto Ciências Biomédicas Abel Salazar (ICBAS), Univ. Porto; ³ Unidade Corino de Andrade (UCA), Centro Hospitalar do Porto (CHP), Porto, Portugal; ⁴ Center for Neuroscience and Cell Biology (CNC), Univ. Coimbra; ⁵ Faculty of Medicine, Univ. Coimbra (FMUC), Portugal
diana.santos@ibmc.up.pt

INTRODUCTION: Familial amyloid polyneuropathy (FAP ATTRV30M) is an autosomal dominant systemic amyloidosis caused by misfolded and aggregation of mutated transthyretin (TTR). The most frequent mutation in the TTR gene (chr18q12.1), V30M, is associated with several clusters. Among Portuguese families, FAP shows a wide variation in age-at-onset (AO) [19-82 yrs.] and this variability is also evident between generations. Importantly, significant differences in AO regarding gender are known in Portuguese series, where women were found to have a later-onset than men and where larger anticipation (>10 yrs.) was more often inherited from mothers (70%) than from fathers (30%). Our aim was then to quantify the mitochondrial DNA (mtDNA) copy number in a FAP Portuguese sample in order to unravel the involvement of mtDNA in FAP mechanisms.

MATERIAL & METHODS: We analysed a total of 265 DNA blood-derived samples from 58 early-onset (≤40 yrs.) and 53 late-onset (>40 yrs.) patients, 67 asymptomatic carriers (>40 yrs.) and 30 non-carriers individuals (belonging to same familial background). Healthy controls (n=57) without any FAP familial history were also included in the study. Quantification of the mtDNA copy number was performed by quantitative real-time PCR and statistical analysis was assessed using SPSS v.23 software.

RESULTS: Comparison of different groups mentioned above yield that the highest median of mtDNA copy number was observed in early-onset patients, although patients and asymptomatic carriers have higher median mtDNA content than controls. Regarding parent-offspring pairs analysis, we found a significant increase in the mtDNA copy number in the early-onset offspring, when compared with their affected parents (p=0.004). Additionally, we also found significant differences in the female group when we compared late- and early-onset patients. Female early-onset patients have higher median mtDNA content than female late-onset patients (p=0.018).

DISCUSSION & CONCLUSIONS: These findings revealed, for the first time to our knowledge, that mtDNA copy number is associated with earlier FAP ATTRV30M events. Moreover, it is known that as more misfolded proteins are produced, there is extra endoplasmic reticulum stress possibly leading to increase in reactive oxygen species (ROS) production. Thus, we hypothesized that an adaptation of the energetic metabolism must have occurred in these patients through a compensatory mechanism, involving an increase of ROS and consequently energy failure. Therefore, our results are important to understand the amyloid generating process and may have implications in genetic counselling, as well as in development of novel therapeutic strategies.

INTRODUCTION: Transthyretin (TTR) familial amyloidosis is a rare, fatal disorder caused by mutations of the TTR gene resulting in damaging amyloid accumulation in peripheral and autonomic nerve tissue and various organs. In TTR-familial amyloid polyneuropathy (FAP), the amyloid is primarily deposited in neural tissue. The Transthyretin Amyloidosis Outcomes Survey (THAOS) is an ongoing, international registry of individuals diagnosed with or at risk for TTR amyloidosis. Data obtained from symptomatic subjects with Val30Met and non-Val30Met (non-cardiac) mutations on specific disease-relevant outcome measures were analysed to describe the natural history of TTR-FAP progression.

MATERIAL & METHODS: The analysis (cut-off date: January 6, 2015) included consenting subjects with TTR mutations, excluding those known to be associated with predominantly cardiac phenotypes (Val122Ile, Ile68Leu, Leu111Met, and Thr60Ala), who were symptomatic, and had baseline data and at least one follow-up visit. Subjects who received a disease-modifying treatment before enrollment were excluded. Outcome measures included: 1) the change in derived Neuropathy Impairment Score-Lower Limb (NIS-LL) over time; 2) change in health-related quality of life using the total quality of life (TQOL) score from the Norfolk QOL-Diabetic Neuropathy patient-reported questionnaire; 3) time to disease progression (based on ambulation) from modified Polyneuropathy Disability (mPND) Stage I to II+; and 4) treatment-free survival based on Kaplan-Meier estimates from time of symptom onset and from time of diagnosis. Subjects who received disease-modifying treatment post-enrollment were censored at the earlier date of liver transplant or treatment initiation. Continuous variables were analysed using linear mixed-effect models to predict individual and population mean response trajectories over time with slope representing the average change per year. Categorical variables were summarized with descriptive statistics.

RESULTS: In total, 1429 subjects were included in the analysis, comprising subjects with Val30Met (n=1148) and non-Val30Met (n=281) mutations with median age at symptom onset of 35.3 and 49.1 years, respectively, and median symptom duration of 4.1 years. At enrollment, the predominant phenotype was neurologic (Val30Met, 74.8%; non-Val30Met, 52.3%) and the most common mPND stage was Stage I (able to walk without assistance) (Val30Met, 68.7%; non-Val30Met, 47.1%). The predicted mean change in overall NIS-LL was 1.54 (1.17, 1.92) and 2.66 (1.05, 4.26) points per year for Val30Met and non-Val30Met, respectively. Predicted yearly change in TQOL was 3.24 (2.69, 3.79) and 2.10 (0.95, 3.71) points per year for Val30Met and non-Val30Met, respectively. Of the Val30Met subjects who progressed from mPND Stage I to II+ (n=31), mean (standard deviation, SD) age at progression was 50.9 (14.2) years and mean time to progression was 2.9 (3.1) years. For non-Val30Met subjects (n=8), mean age (SD) at progression was 43.7 (10.8) years and mean time to progression was 3.3 (4.4) years. The 5- and 8-year survival estimates (standard error, SE) for the Val30Met group were: 94.6% (2.6) and 87.7% (4.1) from symptom onset and 87.9% (5.4) and 74.1% (8.6) from diagnosis; for the non-Val30Met group, the 5- and 8-year survival estimates were: 46.7% (17.1) and 34.3% (13.4) from symptom onset and 54.8% (11.8%) and 8.4% (9.5) from diagnosis.

DISCUSSION & CONCLUSIONS: These results from a large cohort of symptomatic subjects in the THAOS registry provide estimations based on genotype of disease progression from symptom onset and survival from both symptom onset and diagnosis. The results can serve as a reference for clinicians monitoring patients and for researchers evaluating the clinical course and management of disease progression.
Global epidemiology of transthyretin familial amyloid polyneuropathy: A systematic review

H Schmidt¹, M Waddington Cruz², MF Botteman³, JA Carter⁴, A Chopra³, M Stewart⁵, M Hopps⁵, S Fallet⁵, L Amass⁵

¹ Universitätsklinikum Münster, Münster, Germany. ² Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. ³ Pharmerit International, Bethesda, MD, USA. ⁴ BluePoint, LLC, Chicago, IL, USA. ⁵ Pfizer Inc, New York, NY, USA.

hepar@ukmuenster.de

INTRODUCTION: Transthyretin familial amyloid polyneuropathy (TTR-FAP) is an irreversible, fatal, and rare autosomal dominant genetic disease characterized by progressive polyneuropathy due to amyloid deposition in the peripheral nerves [1]. Although endemic to Portugal, Sweden, and Japan, TTR-FAP has been found in over 30 countries [2]. TTR-FAP global prevalence has previously been estimated at 5,000 to 10,000 persons worldwide [3,4]; however, this is widely believed to be an underestimate.

MATERIAL & METHODS: Following established systematic review and data synthesis guidelines (Cochrane and PRISMA), a comprehensive search strategy was conducted simultaneously in four electronic databases (EMBASE, PubMed, SCOPUS, and Web of Science). Observational studies published between 2005 and 2015 were included in the review, with older key references (determined by citation frequency) included on a case-by-case basis. A comprehensive database of TTR-FAP epidemiological publications was developed. Key data elements, geographic location, recruitment method, age, gender, genotype, and phenotype were extracted from the literature. Data were also synthesized, where sufficiently available, into global and regional prevalence estimates of TTR-FAP.

RESULTS: Analysis is ongoing and results are expected April 2016. An updated abstract will be submitted at that time. Findings will be stratified by country, by mutation, and by country x mutation where possible.

DISCUSSION & CONCLUSIONS: This systematic review and synthesis of available global epidemiology literature suggests that global TTR-FAP prevalence is approximately <INSERT> per 100,000 persons. These results shed insight on the need for increased disease awareness and early and accurate diagnosis to improve patient management.

Wild-type transthyretin (TTR) amyloidosis of the ureter in a patient with rheumatoid arthritis and Sjögren’s syndrome


Department of Morphological and Physiological Sciences, Graduate School of Health Sciences, Kumamoto University, Kumamoto, Japan. 1First Department of Internal Medicine, University of Toyama, Toyama, Japan. 2Department of Urology, University of Toyama, Toyama, Japan. 3Department of Diagnostic Pathology, Graduate School of Medical and Pharmaceutical Sciences, University of Toyama, Toyama, Japan. 4Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan.

INTRODUCTION
Localized amyloidosis of the genitourinary tract is uncommon and an extremely rare cause of hydronephrosis and renal failure. Monge et al. reviewed 169 reported cases of localized amyloidosis of the genitourinary tract and reported that the involved organs were the bladder (48.5%), ureter (25.4%), urethra (20.1%), and renal pelvis (5.9%) [1]. The disease is almost characterized by AL amyloidosis without systemic disease [2]. Here we present a rare case of ureteral amyloidosis in a middle-aged woman.

METHODS
We investigated clinicopathological and biocharacteristics of a 56-years-old woman with right ureteral amyloidosis associated with rheumatoid arthritis and Sjögren syndrome.

RESULTS
She developed acute pyelonephritis with right hydronephrosis. Although she had successfully been treated with antibiotics, ureteral stenosis sustained. She underwent ureteroscopy and stenting of right ureter. Biopsy specimen revealed submucosal amyloid deposition in the interstitium overlying a benign urothelium. The amyloid protein was neither amyloid A (AA) nor immunoglobulin light chain (AL), but transthyretin by means of immunohistochemistry, and amyloid deposition was not demonstrated in other organs. As the TTR genes were wild type, she was diagnosed with wild-type TTR amyloidosis.

DISCUSSION & CONCLUSIONS:
We should be aware that wild-type TTR amyloidosis can occur at younger age and cause symptomatic ureteral stenosis. Further investigation is needed to clarify the association of autoimmune diseases to develop wild-type TTR amyloidosis.

REFERENCES
**PB47**

**Intraepidermal nerve fiber density as an early diagnostic marker of transthyretin-related familial amyloid polyneuropathy**

T Masuda¹, M Ueda¹, G Suenaga¹, M Tasaki², Y Misumi¹, T Yamashita¹, K Obayashi², Y Ando¹

¹ Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan. ² Department of Morphological and Physiological Sciences, Graduate School of Health Sciences, Kumamoto University, Kumamoto, Japan.

t.masuda0903@gmail.com

**INTRODUCTION:**

Transthyretin (TTR)-related familial amyloid polyneuropathy (FAP) is an autosomal-dominant inherited disorder characterized by systemic accumulation of amyloid fibrils in various organs and peripheral nerves. FAP patients usually show small fiber neuropathy (SFN). Skin biopsy with intraepidermal nerve fiber density (IENFD) is a good diagnostic method for SFN. The aim of this study was to investigate the diagnostic utility of intraepidermal nerve fiber density (IENFD) in the skin biopsies of FAP patients.

**MATERIAL & METHODS:**

We employed 33 TTR-related FAP patients (Val30Met (n= 21), Ser50Ile (n= 2), Gly47Val (n= 2), Tyr114Cys (n= 2), Thr49Ile (n= 1), Thr60Ala (n= 1), Phe33Val (n= 1), Glu89Lys (n= 1), Ala36Asp (n= 1), Ile107Val (n= 1) and 5 iatrogenic amyloid neuropathy (IAN) patients who underwent domino liver transplantations with liver grafts obtained from TTR-related FAP patients. In those patients, we examined clinical features, IENFD, nerve conduction studies (NCS) examined tibial nerve, peroneal nerve, sural nerve, and heat-pain response measured by CASE-IV.

**RESULTS:**

The IENFD was decreased in TTR-related FAP patients (4.5 ± 3.1/ mm). IENFD in FAP patients significantly correlated with FAP clinical score, time from onset, the various parameters of NCS, heat-pain response. FAP patients with the TTR Val30Met mutation showed lower IENFD than FAP patients with non-Val30Met mutations (3.6 ± 2.8 mm in Val30Met, 6.2 ± 3.1 mm in non-Val30Met, p < 0.05). The IENFD was also decreased in IAN patients (3.7 ± 1.8 mm).

**DISCUSSION & CONCLUSIONS:**

The IENFD was decreased both in FAP and IAN patients, suggesting amyloid deposition in the tissues may be related to changes in SF. IENFD in the skin biopsies may be an early diagnostic marker of FAP and IAN patients.
PB48

A late onset case of hereditary transthyretin amyloidosis with a novel compound heterozygous mutation

S Matsumoto, M Ueda, T Yamashita, T Amano, Y Misumi, M Tasaki, T Masuda, M Mizukami, H Furuya, Y Ando

1 Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan, 2 Department of Morphological and Physiological Sciences, Graduate School of Health Sciences, Kumamoto University, Kumamoto, Japan, 3 Department of Neurology, Kochi Medical School, Kochi University, Kochi, Japan

INTRODUCTION:

Hereditary transthyretin (TTR) amyloidosis is characterized by TTR amyloid deposits in various tissue sites and organs, such as peripheral nerves, heart, gastrointestinal tract, kidneys, eyes, and leptomeningeal tissues of the brain. So far, more than 130 mutations in the TTR gene have been identified. Several hereditary TTR amyloidosis patients with compound heterozygous TTR mutations were reported. However, the pathogenesis of those heterozygotes still remains to be understood. Here we report clinicopathological findings of a late-onset case of hereditary TTR amyloidosis with a novel compound heterozygous mutation.

CASE REPORT:

A Japanese man from a non-endemic area of hereditary TTR amyloidosis in Japan developed cardiomegaly at the age of 64. He also developed mild polyneuropathy at the age of 68. TTR amyloid deposits were found in cardiac and gastric biopsies. He did not have symptoms and laboratory data suggesting renal, ocular, and leptomeningeal amyloidosis. Genetic testing and mass spectrometric analysis of serum TTR revealed that he had a novel compound heterozygous TTR Val30Met/ Lys80Arg mutation. His elder brother had a heterozygous TTR Lys80Arg mutation and also developed late-onset hereditary TTR amyloidosis with autonomic and sensorimotor polyneuropathy at the age of 70.

CONCLUSION

A novel compound heterozygous TTR Val30Met/ Lys80Arg mutation may cause late-onset of systemic amyloidosis.
Carpal tunnel syndrome: the most common initial symptom of systemic wild-type ATTR amyloidosis

Y Sekijima1,2, M Nakagawa1, M Yazaki1,2, K Tojo1, T Yoshinaga1, J Koyama3, S-I Ikeda1,2

1Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan. 2Institute for Biomedical Sciences, Shinshu University, Matsumoto, Japan. 3Department of Cardiovascular Medicine, Shinshu University School of Medicine, Matsumoto, Japan.

sekijima@shinshu-u.ac.jp

INTRODUCTION:
Systemic ATTRwt amyloidosis is a prevalent aging-related disorder, as 12% – 25% of people over the age of 80 and 37% of people over the age of 95 were shown to have ATTR deposition in postmortem studies1-3. However, a limited number of systemic ATTRwt amyloidosis patients have been diagnosed antemortem, and therefore the prevalence of ATTRwt is underestimated. In this study, we analyzed the clinical, radiological, and pathological findings of a series of systemic ATTRwt amyloidosis patients with antemortem diagnosis and discuss indicators for the early diagnosis of this disease.

MATERIAL & METHODS:
Thirty-one consecutive patients diagnosed with systemic ATTRwt amyloidosis at Shinshu University Hospital were included in this study. Systemic ATTRwt amyloidosis was diagnosed based on proven ATTR amyloid deposition in biopsy specimens and confirmation of wild-type TTR genotype. Patients with localized tenosynovium ATTRwt amyloidosis were excluded from the study. We performed diagnostic interviews with patients and their family members very carefully at the first medical examination and followed up at least once a year.

RESULTS:
The systemic ATTRwt amyloidosis patients consisted of 24 men and 7 women (male-female ratio, 3.4:1), and mean age of onset was 69.8 ± 9.0 years. The most common initial symptom was carpal tunnel syndrome (17 patients, 54.8%), followed by heart failure symptoms (14 patients, 45.2%). The mean age at diagnosis was 74.5 ± 8.3 years and the duration of illness from onset to diagnosis was 5.4 ± 4.4 years. 99mTc-PYP myocardial scintigraphy was performed in 24 patients and abnormal uptake was observed in 23 patients, including two patients who did not have cardiac symptoms or signs.

DISCUSSION & CONCLUSIONS:
Carpal tunnel syndrome is the most common initial symptom of systemic ATTRwt amyloidosis. Our results suggest the possibility of systemic ATTRwt amyloidosis diagnosis at an early stage by carefully examining patients with carpal tunnel syndrome4. 99mTc-PYP myocardial scintigraphy is very sensitive in detecting cardiac ATTR amyloidosis.

REFERENCES:
PB50

Bortezomib and dexamethasone (BD) followed by risk adapted high dose melphalan and autologous stem cell transplantation (RA-SCT) or RA-SCT followed by BD consolidation in newly diagnosed transplant-eligible patients with AL Amyloidosis

H Landau, C Sarubbi, S Devlin, R Comenzo, S Giralt, O Landgren, Hassoun.

INTRODUCTION: The depth and durability of hematologic response is a critical determinant of outcome in patients (pts) with light chain (AL) amyloidosis. Complete hematologic remissions (CR) following risk-adapted melphalan and stem cell transplant (RA-SCT) in pts with AL is associated with organ improvement and extended overall survival (OS). We have previously shown that using bortezomib and dexamethasone (BD) consolidation following RA-SCT is associated with deep hematologic responses and favorable outcomes. The current prospective phase II trial uses BD as induction followed by RA-SCT and BD consolidation in newly diagnosed, transplant-eligible AL pts.

MATERIAL & METHODS: Untreated pts with AL amyloidosis received 1-3 cycles of BD (B 1.3mg/m2, IV/SC, and D 40mg, IV/PO, days 1, 4, 8, 11). BD was discontinued before 3 cycles in patients who achieved CR. Pts were then assigned melphalan 100, 140 or 200mg/m2 based on age, renal function and cardiac involvement; 3 months (mos) following RA-SCT, pts received six cycles of BD (B 1.3mg/m2, IV/SC and D 20mg, IV/PO days 1, 8, 15, 22) every 12 weeks as consolidation. Hematologic and organ responses were assessed using International Society of Amyloidosis and updated organ response criteria, after induction, 3 mos post RA-SCT, and at 12 mos from treatment initiation. Pts with NYHA Class III/IV heart failure, ECOG > 2 or > grade 2 neuropathy were ineligible.

RESULTS: Twenty pts, 70% male, with a median age of 60.1 years with renal (55%), cardiac (65%), liver/GI (15%) or nervous system (15%) involvement received BD induction and 18 pts have been transplanted. Two pts with cardiac disease died during BD induction (10% TRM); 85% of pts are alive with a median follow up of 28 mos. By intent to treat (ITT), 60% and 70% of patients achieved at least a very good partial response (VGPR) following BD induction and RA-SCT, respectively. Overall, 95% of pts achieved hematologic responses (PR) including 35% CR. Cardiac and renal responses were seen in 58% (7/12) and 77% (10/13) of evaluable pts. Grade >3 adverse events included GI (40%), renal (30%), infectious (10%), and cardiovascular (10%); Grade >2 neuropathy was seen in 40% of pts and warranted discontinuation of BD in 35% of pts.

DISCUSSION & CONCLUSIONS: In newly diagnosed AL pts, BD induction followed by RA-SCT was safe and rapidly and effectively induced responses resulting in organ improvement. There was 10% TRM during BD induction and no deaths during transplant supporting the notion that early mortality in newly diagnosed AL pts is independent of treatment received. The high incidence of neuropathy maybe related to the administration of BD on a twice-weekly schedule and rendered some pts ineligible for post-transplant therapy. In contrast to our prior study where we observed a marked deepening of response with sequential therapy by ITT (CR following upfront RA-SCT 28%; CR following RA-SCT then BD 56%), the depth of response did not significantly increase when RA-SCT followed BD induction. Whether transplant-eligible pts will ultimately derive more benefit from proteasome inhibitor induction versus consolidation is worthy of further study.


Table 1. Hematologic responses with sequential treatment programs.

<table>
<thead>
<tr>
<th>ITT (N=20)</th>
<th>Following BD</th>
<th>Post RA-SCT</th>
<th>12 mos Post Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall response rate</td>
<td>90% (18/20)</td>
<td>85% (17/20)</td>
<td>85% (17/20)</td>
</tr>
<tr>
<td>CR</td>
<td>20% (4/20)</td>
<td>20% (4/20)</td>
<td>35% (7/20)</td>
</tr>
<tr>
<td>ITT (N=40)</td>
<td>Post RA-SCT</td>
<td>Following BD</td>
<td>12 mos Post Treatment</td>
</tr>
<tr>
<td>Overall response rate</td>
<td>45% (18/40)</td>
<td>85% (34/40)</td>
<td>85% (34/40)</td>
</tr>
<tr>
<td>CR</td>
<td>28% (11/40)</td>
<td>56% (22/40)</td>
<td>56% (22/40)</td>
</tr>
</tbody>
</table>
Heavy/light chain analysis in 200 newly diagnosed patients with AL amyloidosis

Johan Bijzet, Hans Nienhuis, Ingrid I. van Gameren, Anneke C. Muller Kobold, Jacobus G. van der Belt, Bouke P.C. Hazenberg

Departments of Rheumatology & Clinical Immunology and Pathology & Laboratory Medicine, University Medical Center Groningen, University of Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands
h.l.a.nienhuis@umcg.nl

INTRODUCTION: Detection of a free light chain lambda or kappa (the precursor of AL amyloid) or an M-protein is important in the evaluation of AL amyloidosis. The quantitative free light chain (Freelite®) assay has dramatically improved detection and follow-up of patients with AL amyloidosis.

Objective: To study additional utility of the quantitative heavy/light chain (Hevylite®) assay to the free light chain assessment for detection of AL amyloidosis.

MATERIAL & METHODS: Serum from 200 consecutive patients with AL amyloidosis (104 men and 96 women, median age 63, range 33-88 years) was studied using first the free light chain assay followed by the heavy/light chain assay. Results were compared with serum protein electrophoresis, immunofixation, and urine electrophoresis.

RESULTS: Hundred and nineteen patients were diagnosed AL-lambda (both increased free lambda concentration and decreased k/l ratio) whereas 53 patients were diagnosed AL-kappa (both increased free kappa concentration and increased k/l ratio). Ten (5%) of the remaining 28 patients (14%) had a quantifiable heavy/light protein (6 IgG-lambda, 1 IgG-kappa, 1 IgA-lambda, 1 IgA-kappa and 1 IgM-kappa) with both increased concentration and abnormal k/l ratio. Identical M-proteins were found in these 10 patients using serum immunofixation. Eighteen patients (9%) were not identified using the quantitative free light chain and heavy/light chain assays. Ten of these 18 patients had an M-protein or light chain using serum immunofixation (5 IgG-lambda, 2 IgA-lambda, 1 IgM-lambda, 1 IgG-kappa, and 1 BJ-lambda) and 11 had a light chain or M-protein using urine immunofixation (9 BJ-lambda, 1 IgA-lambda, and 1 IgG kappa). No M-protein or light chain was detected in 5 patients (2.5%) whatever detection method was used.

DISCUSSION & CONCLUSIONS: Quantification of free light and heavy/light chains is useful to detect 91% of AL patients, though qualitative detection using immunofixation of serum and urine is still necessary to identify another 6.5% of AL patients.
PB52

Indirect ELISAs for lambda and for kappa free light chain quantification in fat tissue of patients with AL amyloidosis

E. Eelsing, J. Bijzet, B.P.C. Hazenberg

Department of Rheumatology & Clinical Immunology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands

j.bijzet@umcg.nl

INTRODUCTION: Fast and reliable typing of amyloid is essential for prognosis and treatment of a patient after amyloid has shown to be present. Immunohistochemistry is helpful for correct typing of amyloid in biopsies, but frequently fails to distinguish unambiguously between AL and ATTR type. Guanidine extraction of amyloid from fat tissue enables immunochemical detection and quantification of the characterizing protein. This approach has been successful in typing of AA and ATTR amyloid (1, 2). Apart from typing, the quantitative results may reflect the severity of amyloid deposition in tissue during the course of the disease. Therefore two indirect ELISAs were developed for lambda and for kappa free light chain, their diagnostic performances studied for typing of AL amyloid, and the quantitative results related to the semi-quantitative grading of amyloid in Congo red-stained fat aspirates.

METHODS: Subcutaneous abdominal fat tissue aspirates of 197 consecutive patients with AL amyloidosis were studied. The AL subtype was lambda in 143 patients and kappa in 54 patients. The results were compared with those of 209 controls (27 AA, 34 ATTR, 2 AApOAL, 1 Aβ2M, 29 localized amyloidosis and 116 non-amyloidosis controls). The amount of amyloid in fat tissue was graded semi-quantitatively in Congo red-stained specimens; 0 (no amyloid), 1+ (<1% of inspected area), 2+ (1-10%), 3+ (10-60%), 4+ (>60%). A minimum of 30 mg of fat tissue per patient was aimed for to be used for extraction of the amyloid in 1 ml of a 6 M guanidine solution. Free lambda and kappa light chain concentrations were measured using two newly developed ELISAs for lambda and kappa each. Microtiter plates (Corning) were coated overnight with samples, diluted 1:200 – 1:25000. Rabbit anti-human lambda or kappa free light chains (Dako, 1:500) were used in combination with goat anti-rabbit Ig-HRP (Dako, 1:1000), followed by a color reaction with TMB. The controls were used to calculate the 99% one-sided upper reference limits for kappa and for lambda and the 99% two-sided reference limits for the ratio of both light chains.

RESULTS: The intra-assay variability coefficients for lambda and kappa were 7.5% and 8.6% and the inter-assay variability coefficients were 9.7% and 9.4%, respectively. The limit of detection was 4.66 ng/ml for lambda and 3.34 ng/ml for kappa and the lower limit of quantification (LLOQ) was 22.2 ng/ml for lambda and 13.8 ng/ml for kappa. In case of 30 mg fat tissue, the LLOQ was 0.74 ng/mg fat for lambda and 1.10 ng/mg fat for kappa. The lower and upper 99% reference limits for the kappa/lambda ratio were 0.09 and 10.2. As reference area was chosen an area defined by the upper limit of 1.10 ng/mg fat for kappa and the upper limit of 1.32 ng/mg fat for lambda and a kappa/lambda ratio between 0.09 and 10.2. Eleven controls had values outside this reference area, resulting in a specificity of 95% (95% CI is 91-97%). Sensitivity was 7% (1 of 14) for the 0 graded group of AL patients, 33% (9 of 27) for the 1+ group, 63% (15 of 24) for the 2+ group, 90% (53 of 59) for the 3+ group, and 99% (72 of 73) for the 4+ group. In abundant amyloid (3+ and 4+) sensitivity is 95% (95% CI is 89-98%). Concordance was seen between the median values of both kappa and lambda and the grade of amyloid.

CONCLUSION: Immunochemical measurement by ELISA of lambda and kappa free light chain concentrations in fat tissue is a fast and useful method for typing AL amyloid in patients with systemic amyloidosis. The specificity is high and the sensitivity is determined by the grade of amyloid: sensitivity is high in abundant amyloid (3+ and 4+), moderate in little amyloid (2+) and low in minute amyloid (1+).

A restrictive pattern of LV filling is only present in one third of cardiac AL amyloidosis patients

S. Perlini1,2, P. Milani2, L. Obici2, F. Salinaro1, R. Mussinelli1, F. Musca1, G. Gioia1, G. Rizzola1, G. Palladini2, G. Merlini2

1Clinica Medica 2 Dept. Internal Medicine, and 2Amyloidosis Research and Treatment Center, Dept. Molecular Medicine, Foundation IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy
stefano.perlini@unipv.it

INTRODUCTION: Cardiac amyloidosis represents an archetypal form of restrictive heart disease, characterized by profound diastolic dysfunction, associated with a “normal” ejection fraction (EF) that is preserved until the late stage of the disease in the vast majority of patients. Therefore cardiac amyloidosis patients typically fulfill the definition of heart failure with preserved ejection fraction (HfPEF). A restrictive pattern of transmitral left ventricular (LV) filling is often reported as a typical hallmark of diastolic dysfunction in these patients.

MATERIAL & METHODS: In order to evaluate the extent of diastolic dysfunction in cardiac light-chain (AL) amyloidosis, we enrolled 221 consecutive previously untreated subjects (mean age 64 ± 10 years), in whom a first diagnosis of cardiac AL amyloidosis was concluded between 2009 and 2012, according to the International Society of Amyloidosis criteria. Further inclusion criteria were EF>50%, and the absence of significant valve disease, previous myocardial infarction, atrial fibrillation, or chronic obstructive lung disease. The extent of diastolic dysfunction was graded according to the ESC guidelines. To this aim, transmitral Doppler early (E) and atrial (A) velocities, E deceleration time, pulmonary venous flow velocity, early diastolic tissue Doppler peak velocity (E’) and E/E’ ratio were recorded at diagnosis. Survival was assessed over a median follow-up of 35.8 months (range, 19-60 months).

RESULTS: New York Heart Association Class was III or IV in 149/221 cardiac AL patients. As to extracardiac organs, renal, hepatic, soft tissue, peripheral nervous system, and gastrointestinal involvement was present in 65%, 17%, 17%, 14%, and 4% patients, respectively. Quite surprisingly, grade III diastolic dysfunction was only present in 37.1% of the whole cardiac AL population (82/221), grade II and grade I diastolic dysfunction being evident in 84 (38.0%) and 55 (24.9%) patients, respectively. The extent of amyloid deposit, as assessed by interventricular septal thickness, was slightly lower in grade I than in grade III diastolic dysfunction groups (14.2±2.0 vs. 14.7±2.1 mm; p<0.05). Both left atrial dimensions and estimated systolic pulmonary pressure progressively increased from grade I to grade III diastolic dysfunction (p<0.01 for both). At variance with EF, the grade of diastolic dysfunction was a significant predictor of survival after a 3-year median follow-up (p<0.001).

DISCUSSION & CONCLUSIONS: A clear-cut restrictive LV filling is only present in one third of patients with overt cardiac AL amyloidosis, grade I diastolic dysfunction being present in almost one fourth of patients. Despite being an important prognostic factor, the presence of a restrictive pattern of transmitral LV filling cannot be viewed as a “red flag” diagnostic marker in cardiac AL amyloidosis.
PB43

The majority of patients with relapsing light chain (AL) amyloidosis are not eligible for enrollment onto clinical trials: using screen failures to define major unmet medical need

1H Landau, 2R Comenzo, 1T Balasinorwala, 2M Warner, 1H Hassoun.

1Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY.
2Department of Medicine, Tufts University Medical Center, Boston, MA.
Landauh@mskcc.org

INTRODUCTION: Hematologic response criteria in AL amyloidosis are based on reduction of free light chains (FLCs) and correlate with organ improvement and survival in the front-line setting. Hematologic progression is defined from complete response (CR) as any detectable monoclonal (m) protein or abnormal FLC ratio (FLC must double); and from partial response (PR) as a 50% increase in serum or urine m-protein to > 0.5g/dl or 200mg/d respectively; or a 50% increase in FLC to > 10mg/dl based on consensus criteria; cardiac and renal progression criteria have recently been validated. Trials enrolling relapsed pts define measurable disease by a difference in FLC (dFLC) >5mg/dl such that responses (VGPR, PR) can be assessed. However, many pts with hematologic and/or organ progression fail to meet dFLC > 5mg/dl set by inclusion criteria (if progress from CR) or the high bar of FLC > 10mg/dl set by the progression criteria and are ineligible for clinical trials. Composite criteria for progression involving both hematologic measures and biomarkers of organ damage do not exist. The goal of the current study was to characterize pts with AL and evidence of progression who were ineligible for clinical trials in order to determine the magnitude of this problem and define potential AL study populations whose medical needs are not being met.

MATERIAL & METHODS: Previously treated AL pts screened for clinical trials from 5/2013 to 5/2015 at Memorial Sloan Kettering Cancer Center and Tufts Medical Center were reviewed retrospectively. Trials included 1) phase I/II trial of carfilzomib (NCT01789242), 2) phase I trial of ixazomib (NCT01318902) and 3) phase III trial of ixazomib/dexamethasone versus physician’s choice (NCT01659658). Inclusion for all 3 required relapsed AL with dFLC >5mg/dl and evidence of organ damage. Pts with progressive hematologic and/or organ disease (by consensus or validated criteria) who were screened for these trials were included in this analysis.

RESULTS: Among 36 pts screened, 33% (N=12) enrolled. Yet, 67% (N=24) with hematologic (N=14), cardiac (N =6) and/or renal (N=11) progression were ineligible. Median age was 61 years (range, 41-78); prior lines of therapy were 1 in 38%, 2 in 38% and >2 in 25%. Median BNP, TROP, serum ALB, eGFR and 24hr urine total protein were: 283pg/mL (36-2197), 0ng/mL (0-0.09), 3.4g/dL (1.3-4.8), 66ml/min (7-128) and 1800 mg/24hrs (trace-12,875), respectively. Median involved FLC was 6.48mg/dl (0.93-52.6) and dFLC 4.69mg/dl (0.01-52). 58% (14/24) were ineligible due to dFLC <5mg/dl, which was the most common reason for screen failure despite meeting hematologic and/or organ criteria for progression. Others were excluded for multiple myeloma (N=2), cardiac stage III (N=4), prior malignancy (N=1), number of prior therapies (N=1) and low creatinine clearance (N=2). 92% (22/24) have received therapy: 19 off study, 2 on alternate trials and 1 eventually qualified with dFLC >5mg/dl; 2 are being monitored for FLC progression with unclear clinical implications. One-third of patients ineligible for these trials have died.

DISCUSSION & CONCLUSIONS: The finding that only 1/3 of pts with AL amyloidosis and hematologic or organ progression requiring therapy are eligible for clinical trials demonstrates the limitations of the current definitions of progression and “measurable disease” criteria for enrolling relapsed pts on trials. The necessary decision to treat pts with organ progression in advance of their meeting a criterion for FLC progression (to >10mg/dl) indicates that this arbitrarily defined value needs to be revised. Moreover, time to next therapy rather than progression free survival (as currently defined) is a more relevant clinical trial end point. More sensitive, validated hematologic progression and composite criteria defining progression of hematologic and organ disease are critically needed to identify patients whose level of hematologic disease progression and risk of organ damage is at variance with current criteria as defined by FLCs. This will enable novel therapies that have the potential to reduce the risks of end-stage organ failure and death to be tested in this population.
In vitro and in vivo studies of Systebryl™(PTI-110) : a potential AL amyloidosis therapeutic

L Esposito¹, L. Connors², and K. Duchin¹
¹ProtaMed, Inc., Kirkland, WA, USA. ²Amyloidosis Center, Boston University, USA
ProtaMed, Inc., Kirkland, WA, USA
luke.esposito@protamed.us

INTRODUCTION: Systebryl™ (PTI-110) is a proprietary small molecule that targets specific amyloid proteins, including immunoglobulin (IgG) light chain (LC) amyloid (AL) and the inflammation-associated amyloid A (AA). PTI-110’s therapeutic potential in vivo was first established using a mouse model for AA amyloidosis. In these studies, PTI-110 (25 mg/kg) administered orally for 60 days reduced amyloid deposits in kidney, liver and spleen by >70%, compared to vehicle treated animals. Average plasma concentration of PTI-110 over the dosing interval was approximately 3.88 ng/mL. The potential for PTI-110 as an AL amyloidosis therapeutic was assessed by measuring its anti-aggregation activity using ex vivo AL isolated from a patient with AL amyloidosis, and in vivo nonclinical safety studies were conducted.

MATERIALS & METHODS: Congo red (CR)-positive AL was isolated from the kidney of a patient. Thioflavin T (ThioT) fluorometry and a CR binding assay was used to quantitate levels of AL ex vivo. GLP toxicity studies of 4-week duration were conducted in rats and dogs following various doses of PTI-110 and vehicle via the subcutaneous (SC) route.

RESULTS: In vitro PTI-110 reduces AL fibrils isolated from human kidney in a dose-dependent manner as assessed by the ThioT fluorometry and CR binding assays. After one day of incubation at 0.5 mg/mL amyloid, PTI-110 reduced fibrils by 28% to 86%, over a range of 0.01 to 1.0 (PTI-110 : amyloid; w/w) as assessed by the ThioT assay. The IC50 and IC90 values were approximately 0.2 mM (0.05 mg/mL) and 2 mM, respectively. Complementary assays (CR binding and Thioflavin S) and electron microscopy confirmed the disaggregation of the fibrils. Nonclinical GLP 4-week safety studies have shown that the no observed adverse effect level (NOAEL) in rats and dogs after SC administration occurred at peak levels (Cmax) of 16,100 and 501 ng/mL, respectively. Area under the plasma concentration curves from 0 to 24 hours (AUC) were 27400 and 772 ng*h/mL, respectively and at the highest non severely toxic dose the Cmax and AUC values in the dog were 955 ng/mL and 2250 ng*h/mL, respectively. At the NOAEL, where mild and reversible toxicity occurs, the average concentration of PTI-110 was 32.2 ng/mL in dogs (the more sensitive species vs. rat), about 8 times higher than levels that were associated with reduction in amyloid fibrils in the AA model (3.9 ng/mL). Although the tissue concentrations of AL fibrils in vivo are not well established, recent studies have shown that concentrations of fibrillar human recombinant variable LC 6 as low as 0.01 µM bind to the cell surface of cultured human cardiomyocytes and result in metabolic dysfunction (1). Thus, even at concentrations of 0.01 µM PTI-110 may bind to and remodel fibrils that would otherwise adhere to the cell surface and thereby prevent toxicity to cardiomyocytes.

DISCUSSION & CONCLUSIONS: PTI-110 can both inhibit AL formation as well as disrupt and reduce AL once it has formed, thereby representing a potential advantage over therapeutics that target only a specific form of AL. The PTI-110 levels required to disaggregate fibrils in vivo in mice were considerably less than those at the NOAEL in dogs. These data also suggest that the in vitro experiments, using fixed amounts of amyloid at relatively high concentration (compared to in vivo), may underestimate the potency of the anti-amyloid effects of PTI-110 in vivo.

REFERENCES:
Renal outcomes of autologous stem cell transplantation among patients with light-chain amyloidosis: a single centre Spanish experience

SE Azorín1, MT Cibeira2, M Solé1, C Fernández de Larrea2, L Rosiñol2, M Rovira2, JM Campistol1, J Bladé2

1 Department of Nephrology. 2 Department of Hematology. 1 Department of Pathology. Amyloidoses and Myeloma Unit, Hospital Clinic, Institut d’Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, Barcelona, Spain.
azorin@clinic.ub.es

INTRODUCTION: Autologous stem cell transplantation (ASCT) is the therapy of choice in systemic light-chain (AL) amyloidosis, a disease in which the kidney is involved in more than two-thirds of patients at diagnosis [1]. Although prognosis is mandated by heart involvement, when amyloidosis is systemic and affects the kidney, substantial morbidity arises complicating further the management of disease, outcome and quality of life in this fragile population [2]. Recently, on based estimated glomerular filtration rate (eGFR) and proteinuria, a “renal risk” staging system was proposed and validated by Palladini et al in order to better predict at treatment initiation and after 6 months the risk of dialysis necessity at two years [3]. We aim here at describing our experience of renal function evolution in a retrospective cohort of patients with AL amyloidosis undergoing ASCT at our institution.

MATERIAL & METHODS: Basal demographic and clinical characteristics were collected retrospectively from a cohort of 59 patients with a proven diagnosis of AL amyloidosis who underwent ASCT between November 1997 and September 2015 at our institution. Renal staging of patients and renal response were assessed in this population in accordance with Palladini’s study (stage I: proteinuria ≤5g/24h and eGFR ≥50 mL/min/1.73m2; stage III: proteinuria >5g/24h and eGFR<50 mL/min/1.73m2; stage II: neither fitting stage I nor III; renal response at six months: a decrease in proteinuria by ≥30% or below 0.5 g/24 h without ≥25% decrease in eGFR determined using the CKD-EPI formula.

RESULTS: In our series, median eGFR and 24h proteinuria at baseline were 66.20 mL/min/1.73m2 and 6146mg, respectively, with normal eGFR being present among 13 patients (22%) and physiological proteinuria in 14 (23.7%). Forty-five patients reached 6 months after treatment initiation. Of the latter, 35.6%, 53.3% and 11.1% had been classified as stage I, II and III at baseline respectively with an overall median survival of 62 months (range, 8-192). Eighteen patients died during follow-up, with a median time to death of 70.5 months (interquartile range (IQR) 87.1). Thirty-six patients reached 2 years. Six (16.7%) fulfilled the 6-month criteriae for renal progression (1 stage I, 4 stage II, 1 stage III). Of those, only 3 (50%) necessitated renal replacement therapy (RRT) (two pre-classified as stage II and one as stage III). Thirty did not meet renal progression criteriae, with three patients evolving into dialysis dependence in the latter group. Overall, 6 patients required dialysis [2 out of 5 stage III patients (40%) and 4 out of 18 stage II patients (22.2%)] after a median follow-up time of 65 months (IQR 25.75, range 31-98)] with four dialysed patients (66%) dying after a median 22.25 months (range 9-37) from initiation. No stage I patient necessitated RRT. Eleven patients not needing dialysis (36.7%) died at a mean follow-up of 48.1 months.

DISCUSSION & CONCLUSIONS: Akin to previous data from Palladini et al, our experience on AL amyloidosis patients undergoing ASCT shows that renal staging gives early insight on long-term dialysis risk, with the higher the stage, the worse the renal prognosis. Thus, none of the stage I patients required dialysis and the incidence of dialysis requirement we found among stage II patients is in-between the Pavia and Heidelberg study cohorts. Interestingly, in our cohort, initiation of RRT seems not only delayed beyond the two-year threshold, but also to have a much lower incidence compared to that of the latter series. Also, the fact that only 40% of stage III patients progressed toward dialysis, compared to the 60-85% incidence in the previous Italo-German research, suggests an advantage of ASCT above other therapeutic regimes.

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Rituximab and Bendamustine for the treatment of systemic AL amyloidosis associated with IgM monoclonal gammopathy

A Galinier¹, E Desport¹, D Lavergne², V Javaugue¹, L Ecotière¹, A Rinsant¹, P Allenou Lemaire¹, A Jaccard², F Bridoux¹,³

Departments of ¹Nephrology, CHU Poitiers, ²Hematology, CHU Limoges, ³Centre national de référence maladies rares amylose AL et autres maladies à dépôts d’immunoglobulines monoclonale, France

alienor.galinier@gmail.com

INTRODUCTION:

About 6% of patients with systemic AL amyloidosis have an underlying clonal proliferation of lymphoplasmacytic origin that manifests with IgM monoclonal gammopathy. IgM-associated AL amyloidosis is considered to be associated with poorer prognosis. To date, treatment strategies are still debated and more data are needed to determine if regimens similar to those used in Waldenström’s macroglobulinemia produce better results than current standard regimens. We retrospectively studied the efficacy and tolerance of the combination of Rituximab and Bendamustine (R-benda) in patients with IgM-associated systemic AL amyloidosis.

MATERIAL & METHODS:

Data from 10 patients with biopsy-proven AL amyloidosis and IgM monoclonal gammopathy, who had received R-benda between 2013 and 2015 were extracted from the database of the French reference center for systemic AL amyloidosis. We retrospectively evaluated clinico-biological characteristics, tolerance to treatment, and hematologic and organ responses according to the ISA criteria.

RESULTS:

Median age of the 10 patients at diagnosis was 68 years (58-77) and median follow up was 15.5 months (9-21.5). The hematologic diagnosis was Waldenström’s macroglobulinemia (n=4), B-cell non-Hodgkin lymphoma (n=4) and IgM-MGRS (n=2). The median level of serum IgM paraprotein was 9g/L (8-28), with median free light chain (FLC) level of 52mg/L (29-327). FLC ratio was abnormal in 8 patients (with lambda LC excess in 6), but only 6 patients had a dFLC level>50 mg/L. The median number of involved organs was 1.5 and kidney disease was prominent (n=8) with median serum creatinine level of 90μmol/L (65-164) and median proteinuria of 4.6g/day. Other involved organs were the heart (n=5, median Mayo stage: 2), peripheral nerve (n=4), and liver (n=1). R-Benda was administered as first line in 6 patients (reinforced with Bortezomib in 2 cases), as second line in 3 patients and as third line in 1 patient. Patients received a median of 6 cycles. Hematologic response (HR) was achieved in 7 patients, including complete response (CR) in 2, very good partial response (VGPR) in 2, and partial response in 3. Renal and heart responses were observed in 2 and 4 patients, respectively, all of whom had achieved at least VGPR. Tolerance profile was good, with ≥grade 3 neutropenia in 2 patients, pulmonary infection in 3 patients, and allergic reaction in 1 patient.

DISCUSSION & CONCLUSIONS:

In this small retrospective cohort, hematologic response rate was 70%, higher than observed in earlier studies from the UK National Amyloidosis Center (HR 32%) (1) and from the Pavia Center for Amyloidosis (HR 52%) (2). However, in these studies, patients were recruited from 1988 and 1986 respectively, had received numerous lines of chemotherapy that were based on Rituximab in only in 2/103 and 5/60 patients, respectively. In 2 recent studies from the Pavia and Boston centers, regimens based on the combination of rituximab-bortezomib-dexamethasone, or on the use of rituximab or high dose melphalan and autologous stem cell transplantation, produced HR rates of 78% and 74%, respectively, comparable to ours. R-benda appears as a simple, safe and effective strategy for IgM-associated systemic AL amyloidosis.

REFERENCES:

Outcome of very young (≤ 40 years) patients with immunoglobulin light chain (AL) amyloidosis

JP Abeykoon1, J Paludo1,2, A Dispenzieri1,2, M A Gertz1,2, D Dingli1,2, F Baudi1,2, W Gonsalves1,2, R A Kyle1,2, M Q Lacy1,2, S Hayman1,2, N Leung1,2, T Kourielis1,2, SV Rajkumar1,2, S Kumar1,2 and P Kapoor1,2

1Department of Internal Medicine, 2Division of Hematology, Mayo Clinic, Rochester, MN, USA
abeykoon.jithma@mayo.edu

INTRODUCTION:
AL amyloidosis is the most common form of systemic amyloidosis, with an incidence of approximately 1 case/100,000 person-years in Western countries. The median age at diagnosis is around 64 years. Clinically relevant data with regard to the very young (≤ 40 years) patients are scant.

MATERIALS & METHODS:
Medical records of 3433 consecutive patients with AL amyloidosis who were evaluated at Mayo Clinic, Rochester, MN between 01/01/1995 and 12/31/2015 were reviewed. The clinical characteristics and therapeutic approach of patients, 40 years or younger at diagnosis, were analyzed. Overall survival (OS) was determined using the Kaplan-Meier method.

RESULTS:
Of 3433 patients, 52 (1.5%) were 40 years or younger at the time of diagnosis. The median time between the onset of symptoms and definitive diagnosis was 0.4 years (interquartile range [IQR], 0.25-0.86 years). The median age at diagnosis was 38 years (range: 26-40), with evidence of male and lambda predominance at 60% and 73%, respectively. Weight loss (42%), dyspnea (38%), edema (38%) and fatigue (36%) were the most common symptoms while macroglossia was the most common physical finding (19%) at diagnosis. Unusual initial features included spontaneous splenic rupture (2%) and erectile dysfunction (4%), observed 6-15 months prior to the diagnosis of AL. Forty-two percent of patients had Eastern Cooperative Oncology Group (ECOG) performance status >2 and 27 (52%) patients had involvement of ≥3 organs at diagnosis. Renal involvement was noted in 34 (65%) of patients, and nephrotic range proteinuria was evident in 42% of all patients at diagnosis. For those with cardiac involvement (n=31; 60%), the median NYHA class was 3 (range: 1-4) at presentation. Liver was involved in 16 (31%) patients at diagnosis. The median bone marrow plasma cell (BMPC) percentage was 10 (range: 1%-90%); 6 patients (12%) had concomitant multiple myeloma (AL-CRAB) and 25% had >10% BMPCs without active myeloma (AL-PCMM). FISH results were available in 13 (25%) patients, and of these, 69% had t(11;14). The median follow-up for the entire cohort was 10.5 years (95% CI: 7.4-12.5), and 52% of patients were alive at 10 years (median OS 12.7 years; 95% CI: 4.2-NR) from diagnosis. The median OS for patients diagnosed before the year 2005 (n=28) was 7.6 years (95% CI: 2.4-NR, 1 year OS 71%) versus not reached (95% CI: 8-NR; 1 year OS rate 78%) for those diagnosed after 2005 (n=24); p=0.4. Cardiac involvement was associated with a worse outcome (HR: 3.9; 95% CI: 1.5-11.8; p= 0.004, Figure 1A). Of the 49 patients in whom treatment related data were available, 24 (49%) patients underwent autologous stem cell transplantation (ASCT) upfront (within 6 months of diagnosis). The median OS of these patients at 6-month landmark (LM) from the diagnosis was not reached (NR), 95% CI: NR-NR versus median 5.9 years (95% CI: 0.3,5-12) for those not receiving ASCT upfront; p= 0.0005 (Figure 1B). Six (12%) patients underwent solid organ transplantation (kidney 5, heart 1).

DISCUSSION & CONCLUSION:
AL amyloidosis is rarely encountered (1.5% of all AL cases) at the age of 40 years or below. A substantial delay in diagnosis from the onset of symptoms was noted in our study. Cardiac involvement remains a primary determinant of prognosis in this age-group. Nearly one-half of the very young patients underwent ASCT within 6 months of diagnosis, an approach that was associated with a better outcome. Although over one-half of patients are alive at 10 years from diagnosis, no improvement in early mortality has been evident over the past two decades in this cohort.
Attitudes about when and how to treat patients with AL amyloidosis: an international survey

P Milani\textsuperscript{1,2}, A Dispenzieri\textsuperscript{1}

\textsuperscript{1}Division of Hematology, Mayo Clinic, Rochester, MN, USA; \textsuperscript{2}Amyloidosis Research and Treatment Center, University of Pavia, Pavia, Italy
dispensieri.angela@mayo.edu

INTRODUCTION: The most important aspects of treating patients with AL amyloidosis are making a correct diagnosis as early as possible and finding the best chemotherapy and supportive trials for that individual patient. In 2012, new hematologic response criteria in AL amyloidosis were validated in an international retrospective study. The current definition of hematologic progression is based on the consensus criteria defined in 2005 and are not yet validated. The aim of this survey is to describe the treatment decision making of expert physicians in when and how to treat patients with AL amyloidosis.

MATERIALS & METHODS: Physicians that are member of the International Amyloidosis Society and/or the International Myeloma Society and have a recognized interest in the treatment of AL amyloidosis, were invited to participate with an invitation e-mail. The survey was composed by 6 different sections: initial treatment of AL amyloidosis, hematologic progression (HP), organ progression (OP), treatment strategies and follow-up, attitudes towards subsequent relapses and something about the participants.

RESULTS: Of the 100 physicians who were sent surveys 50 (53\% from Europe and 41\% from USA) completed the survey. The majority of the physician respondents were between the age of 40 and 60 years old (62\%). One-third of the respondents indicated that >50\% of their practice was amyloidosis. Autologous stem cell transplant was considered the first line treatment choice, if medically feasible, by 73\% of the physicians, and cyclophosphamide, bortezomib and dexamethasone regimen is the preferred first line strategy in 72\% of cases. In the non-transplant setting, 62\% treat 2 cycles past their hematologic response goal. Depending on the organ involvement, the goal for initial treatment was CR for 65-72\% and very good partial response (VGPR) for 27-35\%. Waiting to reinstitute therapy until serum dFLC was 50 mg/L for eligibility in clinical trials was considered uncomfortable sometime most of the time by 63\% and 18\% of the participants, respectively. Fifty-two percent said the minimum eligibility for trial entry should be dFLC >50 mg/L, but 37\% felt any abnormality of FLC ratio would be sufficient. In the absence of organ progression but rising FLC, the factors that most influenced when to reinstitute therapy included: the initial dFLC at diagnosis (38\%); how sick the patient was a diagnosis (22\%); and time to FLC rise (18\%). For patients who achieved CR after first line therapy, in the presence of cardiac or renal progression, 37\% and 43\% of providers would consider starting clone directed therapy without evidence of a clone. For patients who achieved VGPR after first line therapy, no change in dFLC was required by 45\% and 39\% of physicians in presence of cardiac or renal OP, respectively. When asked about eligibility criteria for a trial using drug(s) directed at the clone, 52\% felt that measurable hematologic disease without measurable organ dysfunction should be sufficient; 42\% stated that both hematologic disease and organ dysfunction should be required. When asked about eligibility criteria for a trial using drug(s) directed at the amyloid, 67\% felt that measurable organ dysfunction without measurable hematologic disease should be sufficient; 33\% stated that both hematologic disease and organ dysfunction should be required. Information about which criteria physicians were using to document response and how frequently they follow patients, and how they deal with multiply relapsed disease was also collected and will be shared at the meeting.

DISCUSSION & CONCLUSIONS: The aim of this survey was to better understand treatment practices among AL experts. Not unexpectedly, we found that the majority of the experts considered VGPR as a treatment goal in most of the clinical situations. However, the definition of HP is based on subjective clinical judgment, clinical experience and a comprehensive evaluation of the patient status. No agreement was found in the definition of the trigger for the initiation of treatment in the most common treatment scenarios of OP. A consensus statement with the definition and validation of new HP criteria are required, in particular for a better definition of the eligibility criteria in clinical trials for relapsed AL patients.
**Glycosylation of immunoglobulin light chains is associated with amyloidosis.**

P Milani\(^1,2\), D Barnidge\(^3\), D Murray\(^3\), M Kohlhagen\(^3\), G Merlini\(^1\), A Dispenzieri\(^2\)

\(^1\)Amyloidosis Research and Treatment Center, University of Pavia, Pavia, Italy; \(^2\)Division of Hematology, \(^3\)Department of Laboratory Medicine, Mayo Clinic, Rochester, MN, USA

dispenzieria.angela@mayo.edu

**INTRODUCTION:** In light chain amyloidosis (AL), the deposition of immunoglobulin light-chains (LCs) in a fibrillar form could cause irreversible organ damage of the affected organs. The primary structure of LC, including sequence and post-translational modifications (PTM), is thought to be important for the amyloidogenicity of LC. Our group and others have reported that κ and λ exist as monomers and dimers and that glycosylated and cysteinylated forms of the LCs exist in patients with AL. Our group has used high resolution microflow liquid chromatography-ESI-Q-TOF MS (a.k.a. miRAMM) to monitor these PTM’s in LCs from a population of clonal plasma cells. The aim of this study was to evaluate the performance of high-throughput/low resolution MALDI-TOF MS as compared to high resolution/low-throughput microflowLC-ESI-Q-TOF MS to identify LC glycosylation in patients with a plasma cell dyscrasia.

**MATERIAL & METHODS:** Matched serum and urine of 290 patients with physician-ordered PEL, IFE and serum FLC were immunopurified with isotype specific camelid nanobody beads and then analyzed by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). Intact LCs were identified as having a PTM if mass peaks were observed above the normal mass/charge (m/z) range for monoclonal LCs. Samples with an indication for glycosylated LCs by MALDI-TOF MS were reanalyzed by microLC-ESI-Q-TOF MS on a SCIEX TripleTOF 5600 mass spectrometer. Glycosylated LCs were identified by their increased molecular mass and the presence of multiple peaks associated with different glycoforms each separated by the molecular mass of one or more monomers of either pentose (132 Da), deoxyhexose (146 Da), hexose (162 Da), hexosamine (191 Da), N-acetylhexosamine (203 Da), or N-acetyleneuraminic acid (291 Da).

**RESULTS:** Amongst the paired samples of 290 patients, 233 patients had a monoclonal protein by MALDI-TOF-MS and/or IFE. Furthermore, the MALDI-TOF MS mass spectra indicated that 51 of these patients had LCs with a PTM. Of these 51 patients, 63% (32/51) were diagnosed with systemic AL; 14% (7/51) with MM and MGUS respectively, 4% (2/51) had renal failure and a not-confirmed AL amyloidosis and 1 had ALECT2. Of note, 5 patients were identified as M-protein only by MALDI and included 3 renal failure patients, 1 suspect AL, and 1 with ALECT2. The paired samples from the 51 patients suspected of having LCs with a PTM by MALDI-TOF MS were then analyzed by high resolution microLC-ESI-Q-TOF MS. The results showed that 32 of the 51 patients had glycosylated LCs present in their serum, including 1 patient with light chain-MGUS who was suspected, but not proven to have AL, and 1 patient with ALECT2 with a coexistent light chain-MGUS. Analysis of the urine showed that (22/51) patients had glycosylated LCs in urine and the molecular masses of the LCs in urine matched the molecular masses in serum. Six of the 31 patients with glycosylated LC in serum had no monoclonal LC in urine. Eleven additional AL patients (4 untreated) were analyzed by microLC-ESI-Q-TOF MS due to the fact that MALDI-TOF MS indicated the presence of LCs with molecular masses outside the normal range. One was negative for monoclonal LCs and 4 were found to have glycosylated LCs when analyzed by microLC-ESI-Q-TOF MS.

**DISCUSSION & CONCLUSIONS:** We have demonstrated that two-thirds of patients with AL amyloidosis had evidence of PTM of their LC by MALDI and one-third are glycosylated. In aggregate, our data would suggest that patients with AL have higher rates of PTM. These data are too preliminary to delineate sensitivity and specificity for MALDI and/or ESI delineated PTM predicting AL, but it is compelling to note that the non-AL patients who had ESI-defined PTM type patterns included 2 MGUS patients, who had an incomplete evaluation to exclude of AL (i.e. no biopsy done of target organ—heart in one and nerve in the other) and recently diagnosed MM patients who could be at risk for AL over time. In conclusion, we believe that these techniques may be able to increase our ability to identify patients with and/or at risk for AL, but further study will be required.
INTRODUCTION: Renal involvement in AL amyloidosis is common, and results in considerable morbidity and mortality. While many patients with this disease have a diagnostic renal biopsy, there are not well-established techniques for deriving prognostic information from renal biopsies.

MATERIAL & METHODS: In a case control study, 39 patients treated for renal AL amyloidosis had renal biopsies reviewed by an expert pathologist and confirmed by an independent pathologist. Renal injury was assessed using Composite Scarring Injury Score (CSIS) and Amyloid Score (AS). CSIS was derived from the sum of percent global sclerosis and tubulointerstitial fibrosis per high-power field, and averaging all cortical fields. AS was derived from the distribution and extent of renal amyloid. The scores were correlated with baseline characteristics and outcomes: rates of progression to end-stage renal disease (ESRD), renal response, renal relapse in responders, and duration of renal response. We hypothesized that CSIS and AS would correlate with outcomes.

RESULTS: AS increased with the number of organs involved by AL amyloidosis (median AS: 5.0 for 1-2 organs involved, 7.25 for 3 organs, 9.75 for 4 organs, 10.75 for 5 organs, P=0.039). AS also correlated with initial troponin I (Spearman’s rho 0.432, P=0.024). CSIS and AS were similar in patients who underwent autologous hematopoietic stem cell transplantation (n=23) or chemotherapy alone (n=16), those achieving (n=19) and not achieving (n=20) a hematological response, and in patients with (n=19) and without (n=20) cardiac involvement. CSIS and AS did not correlate with baseline proteinuria, serum albumin or brain natriuretic peptide.

Both CSIS (Spearman’s rho 0.359, P=0.025) and AS (Spearman’s rho 0.43, P=0.005) correlated with initial GFR. Compared with patients who did not progress to ESRD (n=29), patients who progressed to ESRD (n=10) had significantly higher median CSIS (5.75 v. 3.30, P=0.048) and AS (9.75 v. 5.5, P=0.01). Receiver operator characteristic curves showed that area under the curve of CSIS and AS was 78.1% and 79.8%, respectively. By Cox regression model, CSIS (β coefficient 4.12, P<0.001) and AS (β coefficient 1.41, P<0.001) were strongly associated with progression to ESRD. Time to dialysis curves for patients with CSIS and AS above and below the median (CS median 3.5, AS median 7.25) are shown in Figure 1. CSIS and AS did not correlate with rates of renal response, renal relapse in responders or duration of renal response.

DISCUSSION & CONCLUSIONS: CSIS and AS correlated with progression to ESRD, demonstrating that these metrics carry both diagnostic and prognostic significance in renal AL amyloidosis. A larger sample size would help determine if CSIS and AS offer predictive value independent of laboratory and clinical variables and to generate a clinicopathologic scoring system. As it is currently difficult to predict which patients will have progressive kidney disease, such a system could offer great clinical utility.
Rapid MALDI-TOF method for detecting and isotyping M-Proteins: evaluation of paired samples of serum and urine in different clinical settings.

P Milani1,2, D Murray3, M Kohlhagen3, D Barnidge3, G Merlini1, A Dispenzieri2

1Amyloidosis Research and Treatment Center, University of Pavia, Pavia, Italy; 2Division of Hematology, 3Department of Laboratory Medicine, Mayo Clinic, Rochester, MN, USA

dispenzieri.angela@mayo.edu

INTRODUCTION: The detection and quantification of monoclonal components (MC) are necessary for the diagnosis and evaluation of response to treatment in plasma cell dyscrasias. The repertoire of tests for identifying a MC includes serum and urine protein electrophoresis (PEL), immunofixation electrophoresis (IFE), and quantitative serum free light chain (FLC). A new assay based on matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) has recently been described by our group. This method can provide detection, quantitation and isotyping data suitable for monitoring patients. In this study we compared the MALDI-TOF-MS method to PEL, IFE of both serum and urine and FLC ratios, for the identification of MC in different clinical settings.

MATERIAL & METHODS: Samples from patients whose physician’s had ordered for PEL, IFE of both serum and urine and FLC in the Clinical Immunology Lab at Mayo Clinic were used. PEL was performed on the SPIFE SPE system (Helena Laboratories) and IFE on Hydrasys 9IF gels (Sebia) and FLC were measured use Binding site assays on a BN-II nephelometer. After disassociating the heavy and light chains by reduction, the five purified samples from serum and five from urine were spotted onto a Bruker Microflex MALDI plate. Automated acquisition (~10 seconds/sample) was performed, and the five LC mass spectra from each enrichment were overlaid.  The presence or absence of the MC was determined by visual inspection.

RESULTS: Paired samples from 290 patients were tested (Table). The median difference of the mass/charge (m/z) distribution between serum and urine was 0.3 daltons (interquartile range: -2, 3.22). Serum MALDI detected 171/172 M-proteins that were identified by s-PEL/IFE; the exception was a newly diagnosed AL patient (though urine MALDI was positive). Urine MALDI detected 145/147 M-proteins that u-PEL/IFE detected; the exceptions were 2 treated patients, one MM and one AL. In both cases that uPEL was negative, the MC was detected by serum MALDI. Using serum testing only, a panel of IFE/FLC outperformed s-MALDI by detecting an additional 12 M-proteins [IFE positive (1), FLC positive (12); 2 MM, 7 AL, 3 MGUS]; however, when the combined results of serum and urine PEL/IFE plus s-FLC were compared to the combined results of serum and urine MALDI, there were only 4 patients that the routine bundles detected over MALDI. These patients had a borderline elevation of the κ/λ ratio (range: 1.81-3.96): one IgAκ treated MM, two MGUS, and a newly diagnosed AL (κ). In contrast, MALDI serum and urine detected M-proteins in 17 patients not identified by PEL/IFE/FLC. Nine of these were treated AL; of interest, 3 of these AL cases had unexplained progressive organs dysfunction despite negative IFE/FLC). The performance of MALDI isotyping was compared to IFE: in serum, agreement in 149/170 (88%); and in urine 115/141 (82%). In 11/21 discordant serum samples and in 3/26 discordant urine samples, MALDI and IFE agreed on the major MC isotype but MALDI identified an additional smaller M-protein. In all the cases, there was LC isotype agreement.

DISCUSSION & CONCLUSIONS: Our study demonstrates that MALDI can be used for detection and isotyping in all the clinical setting investigated and has a comparable sensitivity with PEL and IFE methods.

Table. Patients’ characteristics (N. in parentheses represents previously treated patients).

<table>
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<tr>
<th>Variables</th>
<th>AL/AH1</th>
<th>MM</th>
<th>MGUS</th>
<th>Other PCD2</th>
<th>Renal failure</th>
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<td>N</td>
<td>68 (41)</td>
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<td>58</td>
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<td>+ by SIFE, UIFE, or FLC</td>
<td>52 (27)</td>
<td>88 (65)</td>
<td>59</td>
<td>12 (4)</td>
<td>0</td>
<td>1</td>
<td>212</td>
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<tr>
<td>+ by MALDI S and U</td>
<td>61 (35)</td>
<td>88 (65)</td>
<td>58</td>
<td>12 (4)</td>
<td>4</td>
<td>6</td>
<td>229</td>
</tr>
<tr>
<td>+ by IFE/FLC/MALDI</td>
<td>62 (35)</td>
<td>89 (66)</td>
<td>58</td>
<td>12 (4)</td>
<td>5</td>
<td>7</td>
<td>233</td>
</tr>
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</table>

1Includes 2 AL/AH, 2 localized AL; 2Waldenström’s macroglobulinemia, 9 and POEMS syndrome, 2; 1Non-AL amyloidosis, 7 (5 ATTRwt, 1 ATTrm, 1 ALECT2), non-confirmed AL, 7.
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The anti-CD38 monoclonal antibody daratumumab produces rapid hematologic responses in patients with heavily pretreated AL amyloidosis

GP Kaufman¹, R Witteles², M Wheeler², P Ulloa², M Lughtu², S Arai³, R Lafayette⁴, S Schrier¹, M Liedtke¹

¹Division of Hematology, ²Division of Cardiology, ³Division of BMT, ⁴Division of Nephrology, Department of Medicine, Stanford University Amyloid Center, Stanford, CA, USA

mliedtke@stanford.edu

INTRODUCTION: Immunoglobulin light chain amyloidosis (AL) is a clonal plasma cell disorder in which patients (pts) have significant morbidity and mortality related to amyloid mediated organ dysfunction. While no drugs are FDA approved in the USA for AL, evidence based therapies approved in other plasma cell disorders are frequently used. Novel agent combinations and the selected application of high dose therapy yield high upfront hematologic response rates, but the majority of pts eventually relapse, and response rates in the relapsed or refractory setting remain poor. Daratumumab is a monoclonal antibody targeted to CD38, and was FDA-approved in November 2015 for relapsed/refractory multiple myeloma. There is strong biologic rationale to believe it may have efficacy in other plasma cell disorders which express the CD38 surface antigen, but no results have yet been published in pts with AL amyloidosis. The aim of this retrospective study was to evaluate the efficacy of daratumumab in pts with previously treated AL.

MATERIAL & METHODS: Pts with biopsy-proven AL, followed at the Stanford University Amyloid Center, who received daratumumab were retrospectively studied for evidence of response. Demographic and clinical information including hematologic and organ related labs were obtained from medical records. The research was performed with IRB approval in accordance with the declaration of Helsinki.

RESULTS: Fourteen pts with previously treated AL received a median of 6 doses (range 1-10) of single agent daratumumab at 16 mg/kg weekly for the first 8 weeks, followed by every other week infusions. The median age was 66, and 71% of pts were male. The median number of organs involved by AL was two, with 64.2% and 71.4% of patients having cardiac and renal involvement, respectively. The average number of prior lines of therapy was 3 (max 5) and average time since initial diagnosis was 3 years. Prior therapies included high dose melphalan and autologous stem cell transplantation, bortezomib in combination with cyclophosphamide and dexamethasone; bortezomib, carfilzomib, lenalidomide, pomalidomide or ixazomib with or without dexamethasone; or bendamustine. Of the 14 pts, 10 had light chains evaluable for response by week four. Using consensus criteria¹, we observed 2 complete responses (CR), 3 very good partial responses (VGPR) and 4 partial responses (PR). Of note, 2 of the PRs did not meet criteria for CR based on the presence of IgG kappa on serum protein immunoelctrophoresis (SPIE) which likely represents daratumumab since both pts had negative SPIE prior to daratumumab and one pt has AL lambda. In the 10 evaluable pts, the median difference between the involved and uninvolved light chain (dFLC) decreased from 6.1 mg/dl prior to treatment to 0.55 mg/dl, and seven patients achieved normalization of their involved FLC. Nine pts had infusion reactions to daratumumab with 1 pt having a grade 3 or higher reaction. At the time of this report, no organ responses have been observed.

DISCUSSION & CONCLUSIONS: In a heavily pretreated, single institution cohort of previously treated AL pts, daratumumab produced rapid hematologic responses. Daratumumab should be evaluated in larger prospective studies for efficacy in AL.

Six-minute walk test as a measure of cardiac response in AL amyloidosis

RF Cornell1, I Decker1, SA Goodman1, SE Phillips2, B Concepcion3, S Harrell1, R Hung4, M Jagasia1, A Kassim1, A Langone1, OC Ukaegbu1, SM Rubinstein1, D Slosky4, DJ Lenihan4

Department of Internal Medicine, Divisions of Hematology1, Cancer Biostatistics2, Transplant Nephrology3 and Cardiovascular Medicine4, Vanderbilt University Medical Center, Nashville, TN.

robert.f.cornell@vanderbilt.edu

INTRODUCTION: Cardiac disease represents the most common cause of morbidity and mortality in AL amyloidosis. The 6-minute walk test (6MWT) is used routinely in studies of heart failure, pulmonary hypertension and pediatric sickle cell disease. For example, the MIRACLE and MUSTIC trials demonstrated important therapeutic effects based on an improved 6MWT distance of 39 meters and a change of 23%, respectively. These findings contributed to approval of cardiac resynchronization for the management of heart failure. We hypothesized that improvement in 6MWT distance would correlate with cardiac responses in patients with cardiac AL amyloidosis.

MATERIAL & METHODS: We retrospectively analyzed 22 patients with cardiac AL involvement who underwent 6MWT at diagnosis and at the end of planned initial chemotherapy. Linear regression analysis was used to compare 6MWT distance between patients with a cardiac response versus those without. Spearman’s rho was used to correlate 6MWT with New York Heart Association (NYHA) class, troponin I, brain natriuretic peptide (BNP) and left ventricular ejection fraction (LVEF). Cardiac response was defined as BNP decrease of 30% or NYHA class decrease ≥ 2 in subjects with baseline NYHA class 3 or 4 (adapted from Comenzo, 2012).

RESULTS: All patients received bortezomib (B)-based chemotherapy. Patients received chemotherapy alone (n=10) or B-based chemotherapy followed by AHCT (n=12). Hematological responses (HR) were CR (36%), VGPR (36%), PR (23%) and SD (5%). At baseline, 59% (n=13) had modified AL amyloid cardiac stage I/II and 41% (n=9) stage III. Reassessment of 6MWT occurred a median of 13.5 months from diagnosis. Median percent increase in 6MWT was 26.5% with a median change of 90 meters (range, -120 to 365), p<0.001. 81% (n=18) had improvement in 6MWT distance, 9% (n=2) declined and 9% (n=2) were unchanged (Figure 1A). Fifty percent (n=11) experienced a cardiac response with all patients having a biochemical response and only 2 patients with NYHA response. By linear regression model, a cardiac response resulted in significant improvement in 6MWT distance compared with patients without a cardiac response (p=0.004, Figure 1B). Patients with a cardiac response experienced a median increase of 170 meters of distance traveled by 6MWT when compared with having no cardiac response. HR was not associated with change in 6MWT. By Spearman’s rho, 6MWT distance was not significantly associated with NYHA, troponin I, BNP or LVEF. Median follow-up was 2.15 years (range, 1.10-7.20).

DISCUSSION & CONCLUSIONS: In AL amyloid patients with cardiac involvement, the 6MWT can be used as an objective marker of functional improvement complementing biochemical and imaging parameters of response. Patients experiencing a cardiac response had substantial improvements in 6MWT. Use of NYHA as part of cardiac response is limited by the need for improvement in ≥ 2 classes and in this data set was not additive to biochemical response. Incorporation of the 6MWT to the cardiac response criteria should be considered.
The finding of del 17p in marrow plasma cells from patients with light-chain amyloidosis (AL) may confer a worse prognosis

SW Wong¹, G Palladini², U Hegenbart³, H Landau¹, M Warner¹, A Jaccard³, H Avet-Loiseau⁶, T Hansen⁷, J Blade⁸, MT Cibeira⁸, E Kastritis⁴, A Wechalekar¹¹, A Dispenzieri¹⁰, S Schonland³, RL Comenzo¹

¹John C Davis Myeloma and Amyloid Program, Tufts Medical Center, Boston, MA USA. ²Amyloidosis Research and Treatment Center, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy. ³Amyloidosis Center, University of Heidelberg, Heidelberg, Germany (HDB). ⁴Memorial Sloan Kettering Cancer Center, New York, New York (MSKCC). ⁵Department of Hematology, CHU Limoges, Centre National de Référence Maladies Rares. ⁶The Cancer Research Center of Toulouse, Toulouse, France. ⁷Department of Internal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. ⁸Amyloidosis and Myeloma Unit, Hospital Clínica de Barcelona, Barcelona, Spain. ⁹Department of Clinical Therapeutics, Alexandria Hospital, University of Athens, School of Medicine, Athens, Greece. ¹⁰Division of Hematology, Mayo Clinic, Rochester, MN. ¹¹UK National Amyloidosis Centre, the Royal Free London NHS Foundation Trust, University College London

swong7@tuftsmedicalcenter.org

Survival in AL amyloidosis (AL) is typically determined by cardiac stage and hematologic response to therapy. A role for cytogenetic abnormalities in clonal plasma cells is not well-defined. Recent studies have described possible roles for gain 1q and t(11;14) as prognostic markers, though this remains controversial. In multiple myeloma, del 17p is associated with high-risk disease and a poor prognosis. The effect of del 17p in AL has not been well-studied. We report an analysis of a retrospective multinational study of newly diagnosed AL patients with del 17p.

Newly diagnosed AL patients with del 17p were identified and their clinical characteristics and outcomes summarized. Patients who were found to have AL on myeloma relapse or del 17p at AL relapse were excluded. Methods for determining the presence of del 17p were reviewed. We compared the overall survival (OS) of patients in whom del 17p was identified in < or ≥ 50% of clonal cells, and evaluated the impact of cardiac stage on OS in newly diagnosed patients.

Forty-four patients with newly diagnosed AL and del 17p were identified. Thirty-three cases had del 17p assessed on CD138-selected marrow cells, 8 cases by cytoplasmic immunoglobulin FISH and 3 on marrow mononuclear cells; for the latter, the percentage of marrow plasma cells was used to estimate the del 17p fraction. Commercially available methods were used at all centers. Median age was 65 years and males constituted 61% of cases. Cardiac involvement was present in 72%, 44% of whom were stage 3. Eighty-eight percent had clones of lambda isotype with a median difference between involved and uninvolved FLC (dFLC) of 234mg/L and a median percentage of marrow plasma cells of 20%. Median percentage of clonal plasma cells with del 17p by FISH was 27% (range 2-93%). Seventy-nine percent of cases had del 17p in combination with other cytogenetic abnormalities including t(11;14) in 30%, del 13 in 44% and gain 1q in 16%. Eighty-one percent of patients responded to initial therapy and 39% had ≥ VGPR. Thirty-five percent had a cardiac response to initial therapy.

Median overall survival (OS) for the newly diagnosed AL patients was 45 months. Thirty-one percent had ≥ 50% of clonal cells with del 17p and had a median OS of 34 months while those with < 50% survived a median of 53 months (P = 0.42, Gehan-Breslow-Wilcoxon Test). The median OS for stage 1/2 was 54 months compared to 31 months for stage 3, with a trend towards statistical significance (P = 0.05, Gehan-Breslow-Wilcoxon Test).

We conclude that cardiac stage remains a major determinant of OS; however, 1 patient with 37% del 17p at baseline progressed at relapse to both advanced cardiac disease and plasma cell leukemia, suggesting that del 17p may confer risk in AL as in MM under certain circumstances. We are performing a case-control analysis with a control cohort without del 17p but matched for first line therapy, age, and gender, to further evaluate the role of del 17p in AL.
Impact of cardiac stage and hematologic response on AL amyloidosis patients with renal involvement

SW. Wong 1,2, D. Toskic1, M. Warner 1, A. Moreno-koehler2, D. Fein3, T. Fogaren1,2, CM. Oliver4, SD. Guthrie3, RL. Comenzo1,2

1The John C Davis Myeloma and Amyloid Program, Tufts Medical Center, Boston, MA, USA. 2Division of Hematology-Oncology, Tufts Medical Center, Boston, MA, USA. 3Division of Medicine, Tufts Medical Center, Boston, MA, USA. 4Tufts Medical Center, Boston, MA, USA. 5Prothena Biosciences, Inc., South San Francisco, CA, USA

Cardiac stage and depth of hematologic remission are major predictors of survival for AL amyloidosis patients (1-3). Renal staging in AL amyloidosis has been studied in the context of renal survival (4). Influences on survival for renal patients have yet to be fully defined.

We performed a retrospective study of all AL patients with renal involvement diagnosed at our center between 7/1/08 and 6/30/15. In this cohort of consecutive patients (n=84) median age was 63 (IQR 55-70) and 55% were men. Eighty-six percent had lambda plasma cell disease and median involved FLC was 183mg/L (69-485). Fifty-eight percent had cardiac involvement, 18% GI involvement, and 10% peripheral nerve involvement. Forty-one percent were renal stage 1, 44% stage 2, and 16% stage 3, while 11% were cardiac stage 1, 55% stage 2, and 34% stage 3. Median 24-hour proteinuria and serum creatinine were 5.6g (2.4-10.50) and 1.02 mg/dL (0.80-1.80) respectively, and median eGFR was 72 mL/min (40-90). As first-line therapy, 68% received bortezomib-based regimens and 23% melphalan-based autologous stem cell transplant. Seventy-four percent had a hematologic response. Of those patients with a hematologic PR or better, at 6 and 12 months renal responses occurred in 26% and 36% of evaluable patients respectively while progression occurred in 8% at 12 months. At 6 and 12 months cardiac responses occurred in 29% and 38% of patients respectively. Median overall survival (OS) for this cohort (n=84) was 67 months; those with cardiac involvement (n=49) had a median OS of 37 months. Median OS was not reached for cardiac stage ≤ 2, but was 31 months for those who were stage 3 (P<0.05) (Figure). Median OS was also not reached for patients achieving hematologic response ≥ VGPR with a median follow-up of 17 months.

In conclusion, for AL patients with renal involvement, both cardiac stage and depth of hematologic response were important contributors to overall survival. For patients with hematologic PR or better, responses occurred in 36% of renal patients and 38% of cardiac patients at one year.

Clinical case report of a young patient with multiple myeloma and amyloidosis of multiple organ systems

K Kokoviadou, V Papadopoulos, M Topalidou, A Mpanti, E Papadakis, A Kioumi

1 Department of Hematology, General Hospital Papageorgiou, Thessaloniki

INTRODUCTION:
Up to 30% of Plasma cell dyscrasias (multiple myeloma, waldenstrom macroglobulinemia and monoclonal gammopathy of undetermined significance) or B-cell non-Hodgkin’s lymphoma may have amyloid deposits in vital organs like heart, liver and kidneys. Primary amyloidosis and multiple myeloma involve clonal plasma cell proliferation. The immunoglobulin light chain secreted by the clone lead to its deposition as amyloid because of its physiochemical characteristics. Here we are reporting a case of a young patient with multiple myeloma and amyloidosis of liver, heart, kidneys and gastrointestinal tract.

Case report:
A 45 year old man presented with complaints of progressive weakness and anorexia resulting in weight loss over six months. On physical examination the patient was cachectic with severe bilateral pretibial edema, abdominal distention with a massive hepatomegalie and rectal bleeding. His laboratory tests showed anemia, elevated liver parameter, low serum total protein and albumin and proteinuria on dipstick testing. The patient underwent liver and rectum biopsy, both demonstrate amyloid (amyloid p-component). Additional testing revealed a diagnosis of multiple myeloma (serum and urine protein electrophoreses and immunofixation, serum-free light chain and FLC ratio, bone marrow aspiration and biopsy). The trans-thoracic echocardiography (TTE) showed asymmetric hypertrophy of the left ventricle with abnormal myocardial texture, described as “granular sparkling”. BNP and NT-PROBNP were elevated. The patient underwent also a kidney biopsy, which demonstrate amyloid deposition with Congo red staining. Patient received at the beginning three cycles chemotherapy with VCD (bortezomib-cyclophosphamide-dexamethasone), because of disease progression he received for cycles RAD (lenalidomide-adriamycin-dexamethasone) and after that Pom-Dex (pomalidomide-dexamethasone). Unfortunately, the patient was not a candidate for autologous bone marrow transplantation because of his bad liver and heart function. He continuous the chemotherapy with Melphalan and Dexamethasone with no response, he developed hepatic decompensation and encephalopathy and he died 13 months after diagnosis.

CONCLUSIONS:
Presence of amyloidosis in multiple myeloma patients is usually associated with poor survival. The median survival time in these patients is assumed to be about 6 months and death usually occurs as a complication of amyloidosis effecting major organ systems. Clinicians must consider this diseases when patients present with an extended prodromal illness paired with unexplained multiorgan dysfunction. Maybe an earlier detection could promise a better treatment strategy and overall survival.

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2 Kastritis E, Dimopoulos MA, Recent advances in the management of AL Amyloidosis BJH January 2016 Volume 172, Issue 2, 170 – 186
3 Bahlis NJ, Lazarus HM, Multiple myeloma-associated AL amyloidosis: is a distinctive therapeutic approach warranted? Bone Marrow Transplant 2006; 38: 7-15
INTRODUCTION: Eighty percent of patients diagnosed with amyloid light chain (AL) amyloidosis have elevated serum free light chain (FLC) levels. A dFLC (difference between involved and uninvolved FLC) >180 mg/L is considered a poor prognostic indicator and is included in the Mayo Clinic’s revised staging system. Furthermore, most clinical trials for AL amyloidosis include dFLC >50 mg/L as an eligibility criteria. However, 20% of patients with AL amyloidosis have normal FLCs. Here we report our experience of 100 patients with dFLC <50 mg/L with respect to disease characteristics, treatment and survival.

MATERIAL & METHODS: We performed a retrospective analysis of patients with newly diagnosed AL amyloidosis patients initially evaluated between 2003 and 2013. Patients with a dFLC >50 mg/L and those with a dFLC <50 mg/L who had received prior treatment were excluded.

RESULTS: We identified 100 newly diagnosed AL amyloidosis patients with dFLC <50 mg/L who had not received prior systemic treatment. The clinical features are shown in Table 1. The median survival of these 100 patients has not been reached by Kaplan Meier analysis with a median follow-up time of 6.13 years with range of 0.4-13.3 years (Figure 1).

DISCUSSION & CONCLUSIONS: Patients with a dFLC <50 mg/L represent a small proportion of those newly diagnosed with AL amyloidosis and are thought to have a favourable overall survival. Evaluating hematologic response in these patients following treatment becomes challenging as a very good partial response (VGPR) is categorized as dFLC <40 mg/L. These patients have limited opportunity to participate in clinical trials due to challenges evaluating clinical response; therefore, necessitating better understanding of their clinical features and response to treatment.


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ALBase: an updated and improved platform to study immunoglobulin light chain sequences

A Bhutkar, B Spencer, G Chan, LH Connors, T Prokaeva

Gerry Amyloidosis Research Laboratory, Amyloidosis Center, Boston University School of Medicine, Boston, Massachusetts, USA.

prokaeva@bu.edu

INTRODUCTION: Conventional databases such as GenBank store a wealth of immunoglobulin (IG) sequence data, whereas specialized databases are useful for studying specific types of sequences. VBASE has a directory of all human germline sequences; IMGT®, the most comprehensive resource, consists of fully annotated human IG sequences along with sequences from 150 vertebrates; and IMGT/CLL-DB includes over 21,000 sequences from patients with chronic lymphocytic leukemia. AL amyloidosis presents a unique research challenge as the IG light chain (IGL) proteins comprising tissue deposited fibrils, display extensive sequence variability. For this reason, a large organized database is required to elucidate the links between protein sequence and aggregation. To address these issues, ALBase was created in the Gerry Amyloidosis Research Laboratory at Boston University and made available to the public in 2009. The original database contained 491 amyloidogenic sequences, 213 sequences associated with other plasma cell dyscrasias (PCD), and over 3,200 non-PCD associated sequences; in addition, a collection of functionally productive germline sequences (88 IGL, 68 IGK) were incorporated. Recently, ALBase has been updated to include all amyloidogenic IGL sequences reported since 2009. Moreover, the development and implementation of a computational pipeline ensures that the contents of ALBase remain current.

MATERIALS & METHODS: To maintain ALBase as an up-to-date database, a computational pipeline was created; the conduit allows the database manager to source and parse contents from the IMGT® database. Specifically, the pipeline identifies newly reported sequences that are not present in ALBase, parses alignment information and annotation to generate SQL updates that are subjected to curation, and allows the sequence information to be loaded directly into the database. This improvement facilitates frequent updates to ALBase and ensures user access to the latest information. In addition to sequences sourced from IMGT®, ALBase also includes sequences from GenBank, Uniprot, and the Protein Database (PDB).

RESULTS: Since 2009, 343 amyloidogenic IGL sequences have been added to ALBase. Table 1 summarizes the compiled and available data. Protein sequences (n=102) serve as structural templates to appreciate significantly mutated positions in the context of 3-D conformation. Sequence annotation includes information on the biological nature, respective germ-line gene data, identification of the IGL gene regions, and mutations with respect to donor germline gene. Various sequence retrieval methods such as clinical category, molecular type, cell type, or accession number are available. Individual sequences can be aligned with the donor germ-line genes; multiple nucleotide or protein sequences can be aligned together and downloaded in common formats for analysis.

DISCUSSION & CONCLUSIONS: ALBase contains an extensive compilation of publicly available information on amyloidogenic and other PCD IGL sequences culled from multiple online sources. This specialized, comprehensive, and up-to-date dataset serves as a powerful tool easily accessible to the research community interested in analyses of nucleotide and protein sequences. ALBase can be accessed directly at http://albase.bumc.bu.edu/aldb/search/query or through the IMGT® medical page at http://www.imgt.org/IMGTmedical/Amyloidosis/.

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Table 1. ALBase: IGL sequences by clinical category.
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INTRACTABLE, INOPERABLE, RAPIDLY FATAL κ-CHAIN AL AMYLOIDOSIS, WITH BASOLINGUAL INVOLVEMENT, COMPROMISING RESPIRATION AND DIGLUTITION

A Symeonidis1, V Lazaris1, DI Papachristou1, V Labropoulou1, P Triantafyllou1, E Tzouvara1, M Tiniakou1, A Kourakli1

Hematology Division, Dept of Int. Medicine1, and Dept of Anatomy-Histology & Embryology2, University of Patras, Medical School, Patras, Greece.

INTRODUCTION: We describe an unusually severe case of non-cardiac, non-renal AL amyloidosis/amyloidotic myeloma, in a 66-year old female patient, with progressive clinical course, despite a favourable response to first-line treatment.

PATIENT AND METHODS: This patient reported a previous history of Hashimoto’s thyroiditis-hypothyroidism, and had an otherwise unremarkable previous history. Four months before her admission, she manifested a rapidly-progressing, sublingual-submandibular engorgement, initially considered as a thyroid nodule, but associated with voice changes, tiredness on speech and swallowing disturbances. The patient was hospitalized in the ENT department, and a severe tumorous engorgement of the whole tongue was noticed, following a facial MRI. A biopsy from the basal lingual part revealed deposition of amorphous or slightly fibroid eosinophilic material, which, with the Congo-red staining was strongly positive.

RESULTS: She was referred to our Department with a probable diagnosis of amyloidosis. She was only capable to intake liquid foods. On admission she was anemic (Hb 9.8 g/dl), with moderate leukocytosis-neutrophilia (absolute numbers 12.8 x 10^9/l and 9.2 x 10^9/l, respectively) and thrombocytosis (582 x 10^9/l). Her serum LDH was elevated and serum protein electrophoresis revealed an IgG-kappa M-component. Serum IgG was measured 16.7 g/l, with severe immunoparesis of IgA and IgM (0.08 and 0.07, g/l respectively). Serum free kappa chain was 1530 mg/dl and lambda 5.8 mg/dl (κ/λ ratio 263.8). A 24-hour urine sample disclosed 22.1 gr of kappa chain and 0.055 gr of lambda chain (κ/λ ratio 4018). Her cardiac ultrasound showed normal findings, however, NT-proBNP level was found increased 1127 pg/ml. Her bone marrow examination revealed a diffuse infiltration of abnormal plasma cell population, at about 45%, and the trephine biopsy demonstration κ-light chain- and Congo-red staining positivity, thus confirming the diagnosis of amyloidotic myeloma. She did not complain for bone symptoms and a complete skeletal survey did not reveal lytic lesions. A spinal MRI however, disclosed some findings of myelomatous vertebral involvement. She was treated with the combination of cyclophosphamide-bortezomib and dexamethasone (VCD) q/21 days, and following 3 cycles of treatment, although her serum M-component, IgG and free κ-chain levels were substantially improved, she exhibited dramatic clinical deterioration. She was completely incapable to receive any kind of food, and thus a gastrostomy was performed, to maintain feeding and hydration. Again ENT surgeons considered any surgical intervention unattainable or life-threatening. Soon thereafter, she started to experience inspiratory dyspnea and a treacheostomy was also performed. She then manifested recurrent respiratory and systemic infections from gram+ and gram- microorganisms and she gradually developed renal and respiratory failure, and finally succumbed, with an overall survival of 12 weeks following initial diagnosis or 7 months from the initiation of her disease-related symptoms.

DISCUSSION-CONCLUSIONS: This abstract reports an unusually severe, intractable, inoperable, progressive, and ultimately fatal case of non-cardiac, non-renal, sublingual κ-chain, AL amyloidosis/amyloidotic myeloma, despite a favorable response to anti-myeloma treatment, and implies the need for combination antamyeloma and amyloid-detaching treatment, even in cases with presumably non ominous local consequences.
Evaluating the use of multiparametric flow cytometry in establishing the presence of Minimal Residual Disease in systemic AL amyloidosis: a report of eight patients.

MRE Coyne*1,2, A Baginska*1, D Rowczenio1, AD Wechalekar1

*Contributed equally to this study.

1National Amyloidosis Centre, University College London Medical School, Royal Free Campus, Rowland Hill Street, London, NW3 2PF. 2Mater Misericordiae University Hospital, Department of Haematology, Dublin, Ireland. 3University College Dublin, School of Medicine, Dublin, Ireland. mcoyne@mater.ie

INTRODUCTION: The identification of Minimal Residual Disease (MRD) is becoming increasingly important for defining outcomes in haematological malignancies; particularly in independently predicting prognosis in myeloma. The role of MRD analysis in systemic AL amyloidosis is less well established. Patients with systemic AL amyloidosis at diagnosis have a lower clonal burden than that seen in myeloma. Moreover, upfront autologous transplantation even in the era of novel therapies continues to provide a more durable remission than that seen in myeloma.

MATERIAL & METHODS: Here, as a proof of principle, eight patients with systemic AL amyloidosis in complete response (CR) underwent MRD analysis. MRD analysis was completed if CR was achieved, usually six months from induction treatment. Induction treatments included two patients treated with cyclophosphamide, thalidomide, dexamethasone and six patients with cyclophosphamide, bortezomib and dexamethasone. Two of the six patients that had received CVD based treatment underwent consolidation autologous transplantation.

Bone marrow samples were immunophenotyped with eight-colour multiparametric flow cytometry (MFC). The expression of CD138 and CD38 was used to gate the plasma cell population. Patients were identified as having residual disease if a discreet population of phenotypically aberrant plasma cells comprising ≥ 50 events were identified in the 500,000 event file (0.01% limit of detection). An aberrant phenotype was defined as a lack of CD19 expression, strong CD138 expression, weak CD27 expression, and or weak CD45 expression.

RESULTS: Interestingly, none of the eight patients demonstrated MRD negativity. All patients demonstrated a measurable clone despite evidence of CR, including two patients who had an autologous bone marrow transplantation. CD117 and CD81 expression failed to provide any additional discrimination between polyclonal and clonal plasma cells. Moreover, the spread between CD81 positive and negative populations was difficult to distinguish in certain patients.

CONCLUSIONS: In conclusion, this proof of principle study supports MFC as an effective measurement of MRD in patients with systemic AL amyloidosis. The finding that all patients demonstrate MRD positivity raises difficulties in rationalizing if MRD negativity is necessary for a durable remission in systemic AL amyloidosis. To address this further, an adequately powered study randomizing MRD positive patients to further treatment or no treatment may be necessary.
The Australian Amyloidosis Network: Where are we now and where are we going?

Simon Gibbs1,4, Peter Mollee2,4, Fiona Kwok3,4, Anthony Schwarer1,4, Pat Neely2,4, David Booth3,4, Ming-Wei Lin3,4, Liza Thomas3,4, James Hare1,4, Graeme Stewart3,4

1 – Victorian and Tasmanian Amyloidosis Service, Monash University Eastern Health Clinical School, Melbourne; 2 – Princess Alexandra Hospital Amyloidosis Centre, Brisbane; 3 – Westmead Amyloidosis Clinic, Westmead Hospital, Sydney;
4 – The Australian Amyloidosis Network

INTRODUCTION

Amyloidosis is a diverse group of complex, multi-disciplinary diseases, each with individual, evolving treatments. Three specialist services assisting with diagnosis and treatment of amyloidosis have been established in Australia: Westmead Amyloidosis Clinic, Sydney (WAC est. 2007), Princess Alexandra Hospital Amyloidosis Centre, Brisbane (PAHAC est. 2009) and Victorian and Tasmanian Amyloidosis Service, Melbourne (VTAS est. 2014). These distinct services have now formed into the Australian Amyloidosis Network to enhance collaboration, education and research. We sought to determine the incidence of each amyloid type and availability of novel agents and clinical trials for all amyloidosis types within the Network.

MATERIAL & METHODS

Each centre detailed their services, numbers and characteristics of newly diagnosed patients seen 1st June 2014 and 30th March 2016 and available clinical trials.

RESULTS & DISCUSSION

253 patients were referred and 241 cases of amyloidosis confirmed: 87 at WAC, 69 at PAHAC, and 85 at VTAS.

Breakdown of amyloid types were: AL 98 (40.6%), localized 42 (17.4%), wildtype transthyretin (WT TTR) 38 (15.8%), undergoing typing 21 (8.7%), hereditary 19 (7.9%), AA 11 (4.6%), genetic screening 9 (3.7%), and LECT2 3 (1.2%).

Referral patterns and models of care differ between centres, WAC offers genetic screening and PAHAC laser capture microdissection and tandem mass spectrometry. All access cardiac MRI, bone scintigraphy and provide advice to local physicians. Each centre offers diflunisal and doxycycline/TUDCA for TTR, and participates in the Prothena VITAL study and the ixazomib trial for relapsed/refractory AL. Trials involving antisense oligonucleotides for TTR will open soon. The Network facilitates a co-ordinated approach. A monthly Network teleconference to discuss complex cases is underway and an Amyloidosis Research Group of Australasia has recently formed.

CONCLUSIONS

Approximately 12 new cases of amyloidosis are referred to the Network each month. AL amyloidosis is the most common type, although TTR is frequently seen and needs exclusion to avoid inappropriate chemotherapy. Therapies for non-AL amyloidosis are available, trials of monoclonal antibody therapy are now recruiting and hopefully, trials with antisense oligonucleotides will soon open.
In patients with light-chain (AL) amyloidosis stroke volume index (SVI) and myocardial contraction fraction (MCF) are powerful prognostic echocardiographic measures, independent of cardiac biomarkers staging.

P Milani,1,2 A Dispenzieri,1,3,4, MA Gertz,1, M Maurer,1, F Buadi,1 S Kumar,1, MQ Lacy,1, D Dingli,1, P Kapoor,1, RS Go,1, W Gonsalves,1, S Hayman,1, G Lin,1, YL Hwa,1, N Leung,1, RA Kyle,1, Y Lin,1, G Merlini,2, S Zeldenrust,1, M Grogan5

1Division of Hematology, Mayo Clinic, Rochester, MN. 2Amyloidosis Research and Treatment Center, University of Pavia, Pavia, Italy. 3Division of Biostatistics, Mayo Clinic, Rochester, MN, USA. 4Clinical Cardiovascular Research Laboratory for the Elderly, Columbia University, New York, NY, USA; 5Division of Cardiovascular Disease, Mayo Clinic, Rochester, MN, USA.

dispenzieri.angela@mayo.edu

INTRODUCTION: In AL amyloidosis the presence and the severity of heart involvement is the most important prognostic determinant. Myocardial contraction fraction (MCF), defined as the ratio of stroke volume (SV) to myocardial volume (MV), was proposed as a novel index of myocardial performance. Stroke volume index (SVI), is a routine echocardiographic measure of the amount of blood ejected from the heart in one cardiac cycle, relative to body surface area. It was our goal to assess the prognostic role of MCF and SVI in a large cohort of patients with AL amyloidosis in the context of other prognostic variables.

MATERIAL & METHODS: Patients seen between 4/1/1999 and 2/1/2015 were eligible for this retrospective study if they had an ECHO at the Mayo Clinic, Rochester, MN within 30 days of their AL diagnosis with measurements needed to calculate SVI, EF and MCF. To capture the full cohort (n=754), modeling was initially done excluding Mayo (2012) staging and global averaged left ventricular longitudinal peak systolic strain (LV strain) since limited numbers of patients had these studies, 452 and 238, respectively. Thresholds of continuous variables were chosen using the Contal and O’Quigley method. Hazard Ratios (HR) and survival c-statistics were estimated using Cox proportional hazards.

RESULTS: Among the 754 patients satisfying entry criteria, median age was 64 years (range 32-94) and 65% were male. The best cutoff for SVI was 33 mL/m² (sensitivity: 50%, specificity: 86%) and distinguished two groups with different OS (median survival 51 vs. 6 months, P<0.001). An MCF of 33.6% was the best cutoff predicting survival (median survival 53 vs 8.5 months, P<0.001). The correlation between MCF and LV strain resulted as very strong (rho=-0.85, P<0.001). On univariate analysis the baseline ECHO variables predicting OS were interventricular septum thickness >12 mm (HR 1.9), EF <55% (HR 2.15), SVI <33 mL/m² (HR 2.5), systolic blood pressure (SBP) <95 mmHg (HR 2.25), MCF <33.6% (HR 2.36) and LV strain less negative than -14 (HR 2.7), all with P<0.001. Two different multivariate models showed that MCF and SVI were both independent predictors of survival with EF and biomarkers stages (Table). The c-Statistic of the SVI model was similar to that of the MCF model (0.71 vs 0.70). In the subset of patients evaluable for LV strain, SVI (HR 2.09, P=0.002) was an independent predictor of survival in a model with LV strain (HR 1.8, P=0.07), Mayo 2012 stages III and IV (HR 2.88, P<0.008). In this model, which had a smaller sample size (data not shown), EF and SBP were no longer independent predictors, and the c-Statistic of this entire model was 0.76 (0.71-0.80).

DISCUSSION & CONCLUSIONS: SVI and MCF identified patients with a high mortality risk, and they were independent of the Mayo (2012) staging and EF. An advantage to SVI is that in addition to being independent of LV strain and cardiac biomarker staging, it is routinely obtained in most echocardiographic laboratories and incorporated into software reporting systems. MCF is highly correlated with LV strain (i.e. a “poor man’s strain”), but it requires an additional calculation from routine ECHO variables.

Table. Multivariate models.

<table>
<thead>
<tr>
<th>Model 1</th>
<th>HR (95% CI)</th>
<th>P</th>
<th>Model 2</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVI &lt;33 mL/m²</td>
<td>1.89 (1.40, 2.56)</td>
<td>&lt;0.001</td>
<td>MCF &lt;33.6%</td>
<td>1.54 (1.10, 2.14)</td>
<td>0.01</td>
</tr>
<tr>
<td>EF &lt;55%</td>
<td>1.41 (1.05, 1.89)</td>
<td>0.02</td>
<td>EF &lt;55%</td>
<td>1.58 (1.20, 2.09)</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP &lt;95 mmHg</td>
<td>1.44 (1.07, 1.94)</td>
<td>0.02</td>
<td>SBP &lt;95 mmHg</td>
<td>1.55 (1.15, 2.07)</td>
<td>0.004</td>
</tr>
</tbody>
</table>
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Treatment with bendamustine is associated with a survival advantage in a heavily pretreated population of patients with AL amyloidosis.

P Milani, S Schönland, G Palladini, C Kimmich, M Basset, F Russo, A Foli, S Perlini, T Bochtler, AD Ho, G Merlini, U Hegenbart

1Amyloidosis Research and Treatment Center, Foundation IRCCS Policlinico San Matteo, and Department of Molecular Medicine, University of Pavia, Pavia, Italy; 2Amyloidosis Center, University Hospital Heidelberg, Heidelberg, Germany

paolomilani.pv@gmail.com

INTRODUCTION: Combinations of older drugs and novel agents are constantly improving the outcome of chemotherapy in AL amyloidosis. Bendamustine has demonstrated activity in multiple myeloma and Waldenström macroglobulinemia (WM) and is still in evaluation in a phase II trial in relapsed AL amyloidosis patients (NCT01222260). In the present study, we retrospectively evaluated the safety and efficacy of bendamustine and prednisone in 125 patients with AL amyloidosis treated in two European referral centers.

MATERIAL & METHODS: The databases of the two centers were systematically searched for patients with AL amyloidosis treated with bendamustine and prednisone (BeP). The patients received 28-day cycles of bendamustine (60-90 mg/m² on days 1 and 2) and prednisone (100 mg on days 1-4). Sixty-five patients were treated in Heidelberg (Germany) and 60 in Pavia (Italy). Thirty-five (28%) subjects had WM (IgM monoclonal protein and lymphoplasmacytic bone marrow infiltration) as their underlying disease and received BeP with rituximab (375 mg/m² on day 1, R-BeP).

RESULTS: Median age was 66 years and 77 (61%) patients were man. Heart involvement was present in 73 (58%) of patients and kidney involvement in 70 (56%). Severe cardiac failure, defined as NYHA class III was present in 39 (31%), 22 (18%) patients were classified as stage III according to the 2004 Mayo staging system and 19 (18%) were in renal stage III (only one patient was on dialysis). Seventy-nine (63%) patients were refractory to previous lines of therapy and 12 (10%) received BeP upfront. Median time from diagnosis to treatment initiation was 26 months (interquartile range 8-62 months). Severe (grade 3 or 4) adverse events were observed in 24% of subjects: cytopenia (6%), renal failure (5%), fever/infections (5%), skin rash (4%), intestinal perforation (2%), cardiac failure (2%) portal vein thrombosis, and weight loss, in 1 patient each. By intention-to-treat, 41 patients (36%) achieved hematologic response in a median of 3.7 months, with 2 complete remissions (CR), and very good partial responses (VGPR) in 10 cases (8%). Cardiac responses were observed in 6 of 48 patients with measurable NT-proBNP (13%) and renal response in 7 of the 47 evaluable patients (15%). Amongst 46 patients who were previously exposed to alkylating agents, bortezomib, and lenalidomide, 10 (22%) responded to BeP. Seven of the 13 treatment-naïve patients (54%) responded, with 1 CR and 3 VGPRs. Of the 35 subjects with WM receiving R-BeP, 20 (57%) responded, with 1 CR, and 7 VGPRs. Overall, 68 patients (54%) died. Median follow-up of living patients was 15 months, and median overall survival (OS) from BeP initiation was 21 months. There was no difference in outcome between refractory and relapsed subjects. Patients who obtained at least PR after BeP had a significant survival advantage (median survival 73 vs 23 months, P=0.006). A significant survival advantage was also seen excluding patients who received BeP upfront (median survival 56 vs 20 months, P=0.01) and those with WM (median survival 56 vs 20 months, P=0.03), all landmark analysis at 6 months.

DISCUSSION & CONCLUSIONS: Bendamustine is well tolerated and can be considered as a rescue agent in patients with AL amyloidosis even after exposure to alkylators, immune modulatory drugs and proteasome inhibitors. Promising results were noted in the WM group. Obtaining a hematologic response after BeP was associated with a significant improvement of overall survival also at advanced stages in the course of the disease.
Patients with AL Amyloidosis and low free light chain burden have distinct clinical features and outcome

P Milani, M Basset, F Russo, A Foli, S Perlini, G Palladini, G Merlini

Amyloidosis Research and Treatment Center, Foundation IRCCS Policlinico San Matteo, and Department of Molecular Medicine, University of Pavia, Pavia, Italy
giovanni.palladini@unipv.it

INTRODUCTION: In AL amyloidosis, circulating free light chains (FLC) are not only a clonal marker, but they are the causal agent of the disease. The recently validated criteria of hematologic response are based solely on the measurement of FLC. Patients with a difference between involved and uninvolved FLC (dFLC) <50 mg/L do not have measurable disease and cannot be assessed for response. These patients are commonly excluded from clinical trials. Nevertheless, these subjects represent a significant proportion of the patients suffering from AL amyloidosis, and given the lower burden of the toxic amyloidogenic precursor, we hypothesize that they have a distinctive clinical features.

MATERIAL & METHODS: The study population is composed of 1069 consecutive, newly diagnosed patients with AL amyloidosis evaluated between 2004 and 2015 at the Pavia Amyloidosis Center and prospectively followed for response and survival. We compared the 203 subjects (19%) who had a baseline dFLC <50 mg/L (low-dFLC group) with the remaining 866 patients (evaluable-dFLC group).

RESULTS: In the two groups there was no difference in terms of age at diagnosis, sex, of involved light-chain type. Heart involvement was significantly less common in the low-dFLC group (43% vs. 83%, P<0.001), and cardiac dysfunction was more advanced in the evaluable-dFLC group (Mayo stage III 45% vs 15% P<0.001 respectively). Renal involvement was more frequent (77% vs 63%, P<0.001) and more advanced in the low-dFLC group [renal stage I 35% vs 49% (P=0.01), renal stage II 44% vs 38% (P=0.12) and renal stage III 20% vs 12% (P=0.01)]. After a median follow-up of living patients of 28 months, 57 (28%) and 498 (57%) patients died in the low- and evaluable-dFLC groups, respectively. The 2004 Mayo clinic staging system retained its prognostic significance in both groups (P<0.001). Overall survival was significantly better in the low-dFLC group (median 117 vs. 21 months, P<0.001, Figure 1A). Within each Mayo stage patients with low-dFLC had a longer survival (at 3 years, 87% vs. 76%, P=0.04 in stage I, 68% vs. 43%, P=0.001 in stage II, 37% vs. 21%, P=0.02 in stage III). Complete response was associated with a significant survival advantage in the low-dFLC group (median not reached vs. 117 months, P=0.001, Figure 1B).

DISCUSSION & CONCLUSIONS: Nineteen percent of newly diagnosed patients with AL amyloidosis have very low dFLC burden. They represent a subgroup with a distinct and better outcome compared to other patients. A complete response significantly improved survival in patients with low baseline dFLC, who can thus be included in clinical trials with appropriate stratification and be evaluated for complete response.

Fig. 1A) overall survival of 1069 patients with AL amyloidosis according to dFLC >50 mg/L; 1B) Overall survival of 203 patients with AL amyloidosis with dFLC<50 mg/L according to complete response after first line therapy.
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Predictive values of NT-proBNP and Hepatocyte growth factor in the diagnosis of AL amyloidosis

Arnaud Jaccard, David Lavergne, Estelle Desport, Dania Mohty, Sebastien Bender, Frederica Bompart, Frank Bridoux
French Reference Center for AL amyloidosis, CHU Limoges and Poitiers, France

In AL amyloidosis (AL) a rapid diagnosis before severe cardiac involvement is of paramount importance to improve prognosis. It has been proposed to screen for AL patients with MGUS using NT-proBNP to avoid late diagnosis (1). We have shown that serum Hepatocyte growth factor (HGF) is elevated in AL and could also be used to screen patients at risk (2).

We have compared the serum levels of these 2 bio-markers in patients with AL amyloidosis and in patients with MGUS, to study the selectivity and specificity of both markers in the diagnosis of AL amyloidosis.

NT-proBNP and HGF serum levels were measured with a electrochemiluminescence immunoassay (Cobas®, Roche diagnostics) and a linked immunosorbent assay (Quantikine® R&D Systems), in 76 consecutive patients with biopsy-proven systemic AL at diagnosis and in 67 controls with MGUS with no evidence of AL before any treatment. Median age was 70[39-85] and 69 [41-85] for AL patients and controls respectively, 44 (58%) patients with AL had cardiac involvement, 21% were in stage I, 25% in stage II and 44% in stage III.

The median value of NT-proBNP and HGF was 97 ng/l [50-10924] and 2049 pg/l [1344-4345] in controls and 2389 ng/l [71-49761] and 9151 pg/l [1954-70632] in AL patients respectively (Figure 1). The serum levels of both markers were significantly higher in AL patients than in controls (p<0.001 for NT-proBNP and HGF). The best threshold values to discriminate AL patients and controls were 298 ng/l for NT-proBNP and 2707 pg/l for HGF; 20% and 9% of AL patients had serum levels below these thresholds and 12% and 12% of controls had levels above for NT-proBNP and HGF respectively. The sensitivity and specificity values to predict the diagnosis of AL amyloidosis were 80% and 87% for NT-proBNP and 92% and 88% for HGF.

Both bio-markers seem to be of equal value to screen patients with AL amyloidosis among patients with MGUS. Work is in progress to check the performance of these 2 biomarkers to distinguish among patients with hypertrophic cardiomyopathy, those with AL amyloidosis from those with other types of amyloidosis or with non amyloid cardiomyopathy.


Figure 1
Bortezomib is effective even in previously unsalvageable cardiac AL patients
HI Geller1, A Singh1, JP Laubach1,2, TM Mirto1, DK Dupee1, RH Falk1

1 Brigham & Women’s Hospital Cardiac Amyloidosis Program and 2 Dana Farber Cancer Institute, Harvard Medical School, Boston, MA, USA Higeller@partners.org

INTRODUCTION: AL amyloidosis of the heart is a rapidly progressive disease; if left untreated it has a median survival of 5-6 months. Hematologic response, characterized by normalization of elevated free light chain (FLC) is associated with improved survival; however, the presence of cardiac AL amyloid is considered to be a negative prognostic factor. In 1 series, cardiac AL patients with NT-proBNP >332ng/L and troponin T >0.035 mcg/L had a median survival of 7.1 months despite treatment. Among these patients, defined as severe cardiac amyloidosis, those with an NT-proBNP >8500, had a median survival of only 4.6 months (1). Importantly, this analysis included only 7% patients treated with bortezomib, a proteasome inhibitor recently used for treating AL. Bortezomib offers not only a high rate of FLC responders, but it also appears to produce a more rapid response than previous regimens. We therefore sought to determine whether bortezomib-based therapy provided better survival, particularly when biomarkers are elevated above levels associated with the worst prognosis.

MATERIAL & METHODS: 52 treatment-naïve cardiac AL patients were treated between 2009 and 2015 (24 male, mean age of 63.9 years) with bortezomib-based combinations. Patients also received oral weekly dexamethasone (10-40 mg) and since 2011, most also received cyclophosphamide. Patients were categorized into three groups based on the 3-month FLC response: Group A (normalization of FLC), Group B (>50% reduction but no normalization) and Group C (<50% reduction.). 11 patients died before 3 months and were not included in the results.

RESULTS: 41 patients were analyzable at 3 months: 16 were in Group A, 18 in Group B and 7 in Group 3. Median survival for group A was not reached, was 45.3 months for Group B and 19.8 months for Group C, representing a 2-year survival in each group of 100%, 70.1% and 42.8% respectively. Median survival was significantly higher for CR (group A) vs. others (p=0.003) and between any response (A and B) and non-responders (p<0.03). Compared to survival in the published data, severe cardiac AL amyloidosis (n=34) had a median survival of 33.7 months versus 7.1 months, and for those with NT-proBNP >8500 (n=8), it was 38.8 months versus 4.6 months.

CONCLUSIONS: FLC response rate is high in cardiac AL treated with bortezomib-based regimens (83.7% with >50% reduction, of whom half had a complete response), and correlates with survival. Bortezomib-based therapy may be effective even in the presence of severe cardiac dysfunction and markedly prolongs survival compared with previous therapies. Normalization of FLC is a powerful predictor of medium-term survival (>2 years) and treatment should be aimed at FLC normalization irrespective of the patient’s initial prognostic stage.

REFERENCES
An efficient strategy to produce pathogenic immunoglobulin light chains in mouse allows the modelling of monoclonal Ig deposition diseases but fails to generate AL amyloidosis

C. Sirac1,2, S. Bender1,2, A. Bonaud1, M. Clavel1, M. Aïtamer1, V. Ayala1, F. Bridoux1,2,3, M. Cogné1,4

1-CNRS UMR7276, University of Limoges, France, 2-French national reference center for AL amyloidosis and other monoclonal Ig deposition diseases, 3-Department of nephrology, University hospital, Poitiers, France, 4-Institut universitaire de France

christophe.sirac@unilim.fr

The establishment of reliable animal models is critical to better understand the pathophysiology of diseases related to monoclonal immunoglobulin (Ig) depositions and to set up new therapeutic approaches. To address this issue, we developed transgenic models using site directed insertion of a human Ig light chain (LC) gene (V domain only or complete Ig LC or HC) into the mouse Ig kappa locus ensuring its production by all B and plasma cells [1]. High levels of free LC were achieved after backcrossing with LMP2A mice that feature increased plasma cell differentiation in the absence of Ig heavy chain (HC) production and thus absence of H-L chain association [2]. Thanks to this strategy, the levels of circulating free monoclonal LC obtained were greater than those seen in patients (Table 1). We have established 2 transgenic strains expressing free LC from patients with AL amyloidosis but yet featuring no deposits, highlighting a strong resistance of mice to AL amyloidosis. Strikingly, other mouse models of non-amyloid Ig deposition diseases (HCDD and LCDD), which also feature precipitation and aggregation of monoclonal Ig, proved successful in inducing typical organ lesions and renal dysfunction, faithfully recapitulating the early events of the diseases. Determining why and how mice can resist to AL amyloid formation could provide invaluable tools to better understand AL fibrils formation and to develop innovative therapeutics in human.


Table 1: Serum monoclonal free Ig production in patients and corresponding mouse models

<table>
<thead>
<tr>
<th></th>
<th>λS (AL) patient</th>
<th>λS (AL) mouse</th>
<th>κD (AL) patient</th>
<th>κD (AL) mouse</th>
<th>κF (LCDD) patient</th>
<th>κF (LCDD) mouse</th>
<th>γC (HCDD) patient</th>
<th>γC (HCDD) mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free Ig (mg/L ± SEM)</td>
<td>107 ± 20</td>
<td>494 ± 90</td>
<td>138 ± 38</td>
<td>252 ± 28</td>
<td>ND</td>
<td>1196 ± 138</td>
<td>ND</td>
<td>34.9 ± 7</td>
</tr>
</tbody>
</table>

Figure 1: Immunofluorescence studies on kidney sections of 3 different mouse models. Stainings were obtained using FITC-conjugated antibodies specific for human λ LC (AL), human γ HC (HCDD) or mouse κ LC (LCDD). Note the absence of glomerular deposits in AL mouse (*) but the fluorescent dots in tubules showing an active tubular reabsorption of the transgenic human LC. HCDD and LCDD kidney feature Ig deposits along the tubular and glomerular basement membranes and in the mesangium.
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The Mayo staging score in the era of bortezomib: does it still apply?

A Singh1, HI Geller, JP Laubach1, 2, TM Mirto1, DK Dupee1, RH Falk1

1 Brigham & Women’s Hospital Cardiac Amyloidosis Program and 2 Dana Farber Cancer Institute, Harvard Medical School, Boston, USA.

A Singh11@partners.org

INTRODUCTION: Untreated, the median survival in cardiac AL amyloidosis is 5-6 months. A staging system (“Revised Mayo Score”) showed that survival in treated patients is predicted by troponin T ≥ 0.025 ng/mL, NTproBNP ≥ 1800 pg/mL and a difference between uninvolved and involved light chains ≥180 mg/L, with 1 point for each. With this score, the median survival in treated stage 3 and 4 was 14 and 5.8 months respectively (1). Mayo score patients were almost all treated before the introduction of bortezomib, and bortezomib (a proteasome inhibitor) may be superior to prior therapies (2). We postulated that bortezomib therapy might be associated with a better outcome, and therefore sought to determine the survival, based on Mayo staging, in a group of cardiac AL patients treated with bortezomib as initial treatment.

MATERIAL & METHODS: Outcome was determined in 47 patients, treated with bortezomib as initial therapy for AL amyloidosis involving the heart, from 2009-2015. Patients also received dexamethasone 10-40mg/week, and cyclophosphamide was added in patients treated since 2012. All patients had heart failure optimally treated prior to chemotherapy.

RESULTS: Of 47 patients (mean age 64.3 ±9 years, 24 male), 3 were in Mayo Stage 1 (0 points), 7 in stage 2 (1 point), 12 in stage 3 (2 points) and 25 in stage 4 (3 points). Actuarial survival at 12 and 24 months was, respectively, 100% in stage 1, 100% in stage 2, 75% & 57% in stage 3 and 64% & 54.4% in stage 4. Median survival (Table 1) was over twice as long in stage 3 patients and over 5 times as long in stage 4 patients than survival in treated patients as predicted by Mayo criteria.

DISCUSSION & CONCLUSIONS: Treatment with bortezomib-based regimens results in a much longer survival in patients with cardiac AL amyloidosis than in patients treated with other published regimens. Even among those with severe disease, who previously had a median survival less than 6 months, this therapy prolonged survival to close to 3 years. Treatment of cardiac AL amyloid patients should therefore not be considered futile, and a reassessment of Mayo staging criteria needs to be made, given the marked improvement in outcome in patients treated with proteasome inhibition.

REFERENCES:


Table 1: Observed and predicted median survival

<table>
<thead>
<tr>
<th>Stage</th>
<th>Bortezomib (months)</th>
<th>Mayo predicted (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(0 points)</td>
<td>Not reached</td>
<td>94.1</td>
</tr>
<tr>
<td>2</td>
<td>Not reached</td>
<td>40.3</td>
</tr>
<tr>
<td>3</td>
<td>38.8</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>33.7</td>
<td>5.8</td>
</tr>
</tbody>
</table>
Laryngotracheobronchial Amyloidosis: a clinical and proteomic experience in a single centre

S Mahmood1, J Gilbertson1, N Rendell, S Sachchithananthan1, M Fontana1, T Youngstein, C Quarta1, T Rezk1, CJ Whelan, HJ Lachmann, PN Hawkins, JD Gillmore, AD Wechalekar
National Amyloidosis Centre, Division of Medicine, Royal Free Campus, UCL, London, United Kingdom
s.mahmood@ucl.ac.uk

Introduction/Aims: Laryngo-tracheobronchial amyloidosis is a rare type of localised amyloidosis, characterised by amyloid deposition variously in the upper and lower respiratory tract1. The aims of this study were to characterise the clinical features of patients presenting with laryngeal and tracheobronchial amyloidosis and to conduct proteomic analyses of biopsy samples.

Methods: All patients with laryngeal (n=63) and tracheobronchial (n=34) amyloidosis presenting to the UK National Amyloidosis Centre presenting between January 1980 and December 2011 were studied. Immunohistochemical typing of amyloid was performed on all available biopsies, and more recently laser capture and proteomic analyses were performed 60 available samples. Targeted gene sequencing was undertaken in selected cases and mutations in Apolipoprotein (Apo)AI were sought in all patients with available DNA samples.

Results: Table 1 summarises the characteristics of both groups. One patient diagnosed with laryngeal involvement was heterozygous for apolipoproteinAI Ala160Ser, there was no evidence of other organ dysfunction. Surgical and laser procedures were performed in 39/63 (62%) and 18/34 (52.9%) patients with laryngeal and tracheobronchial amyloidosis respectively, with 29% (n=18) and 24% (n=8) requiring repeated procedures. Four patients (1 laryngeal, 3 tracheobronchial) received radiotherapy with median dose of 20 Gy in 10 fractions, and all achieved symptomatic local control during median follow up of 18.7 (range 6-47) months. Proteomic analysis in 60 patients revealed the 3 signature amyloid proteins SAP, Apo A4 and Apo E in each case; interestingly, all biopsies showed insulin-like growth factor binding protein (IGFBP) complex and notable presence of ApoA1; the former peptide absent in 30 patients with systemic AL (cardiac, renal or liver involvement) and 30 others with wild type ATTR amyloidosis. Analysis of five non-amyloidotic control biopsies suggested that IGFBP and ApoA1 are not normal constituents of laryngeal tissue.

Discussion/Conclusion: Amyloidosis affecting the upper and lower respiratory tract is rare. Primary treatment modalities comprising surgical/laser intervention and radiotherapy may be helpful in needful cases. Insulin-like growth factor binding proteins are involved in somatic growth, cell proliferation, cell transformation and apoptosis, and are considered a key modulator of lung fibroblast proliferation2, which our results suggest may play a role in this disease.

<table>
<thead>
<tr>
<th>Characteristics of 63 patients with laryngeal amyloid</th>
<th>Number (%)</th>
<th>Characteristics of 34 patients with tracheobronchial amyloid</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>29 (46%)</td>
<td>Male</td>
<td>12 (35%)</td>
</tr>
<tr>
<td>Age</td>
<td>57.4 (44.7-66) years</td>
<td>Age</td>
<td>47.6 (54-62.5) years</td>
</tr>
<tr>
<td>Symptom duration</td>
<td>10.5 (6-24) months</td>
<td>Symptom duration</td>
<td>4.3 (8-24) months</td>
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<td>Personal history of cancer</td>
<td>8/56</td>
<td>Personal history of cancer</td>
<td>4/28</td>
</tr>
<tr>
<td>Smoking history</td>
<td>16/56</td>
<td>Smoking history</td>
<td>22/28</td>
</tr>
<tr>
<td>Autoimmune disorders</td>
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<td>3/28</td>
</tr>
<tr>
<td>GORD</td>
<td>6/56</td>
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<td>Details missing</td>
<td>7/63</td>
<td>Details missing</td>
<td>6/34</td>
</tr>
</tbody>
</table>

Location
| Supra-glottic                                         | 6          | Tracheal                                                   | 4          |
| Vocal cord/tonsil                                     | 33         | Right sided bronchial                                      | 11         |
| Subglottic                                            | 16         | Left sided bronchial                                       | 3          |
| Not specified                                         | 8          | Bilateral bronchial                                       | 12         |
|                                                       |            | Not specified                                             | 4          |

Clonality and QALY
| Positive serologic clonality                          | 10         | Positive serologic clonality                               | 10         |
| Quality of life score                                  | 8 (3-10)   | Quality of life score                                      | 5 (1-9)    |

References:
Implantation of intracardiac defibrillators in patients with advanced cardiac amyloidosis

T Rezk, L Carter, F Khan, M Shiu, HJ. Lachmann, M Fontana, PN Hawkins, JD Gillmore, AD Wechalekar, CJ. Whelan

1National Amyloidosis Centre, Centre for Amyloidosis and Acute Phase Proteins, Division of Medicine, Royal Free Campus, University College London, &2Royal Free Hospital, London, UK
t.rezk@ucl.ac.uk

Introduction: Cardiac amyloidosis is a progressive and fatal disease with 30-40% of patients with advanced disease dead within 3 months despite treatment (1). Cause of death as well as trigger for the terminal cardiac event remains unclear. Chemotherapy is challenging as drugs are associated with cardiac destabilization. Previous reports suggested that implantable cardioverter-defibrillators (ICD) had limited impact in reducing mortality (2). Following our recent report of successful treatment of life-threatening ventricular arrhythmia by ICD thrice over 3 years in a patient with immunoglobulin light chain cardiac amyloidosis (AL), we report our experience in 18 patients with cardiac amyloidosis who had an ICD implanted.

Materials and Methods: All patients who underwent ICD implantation at our centre were prospectively followed from 2009 till 2015. Patients underwent serial evaluation every 1-6 months including cardiac biomarkers and echocardiography. Outcomes during follow up were reported.

Results: 18 patients were identified, 15 with cardiac AL and 3 with cardiac transthyretin (TTR) amyloidosis. Median age at diagnosis was 51.8 years with a M:F ratio 2:1. Median Troponin T and NT-proBNP at diagnosis was 60ng/L (range<10 - 326) and 4089ng/L (18.47 - 34,152) respectively. Median time from diagnosis to implantation was 0.427 months (0- 54.51.) 27% of patients with AL had Mayo stage 2 disease, 47% stage 3a and 26% stage 3b. 5/18 patients had evidence of atrial fibrillation on electrocardiogram at presentation with 4/18 patients showing evidence of conduction block. Median left ventricular wall thickness was 15.5mm (9-19) and left ventricular ejection fraction of 53% (40-66.) 18/19 patients underwent ICD insertion for primary prevention with all having evidence of at least non-sustained ventricular tachycardia (NSVT) and 4 who presented with syncope prior to device implantation. 1 patient underwent ICD implantation for secondary (post VF arrest) prevention. 4/18 (22%) patients received therapy from their ICD - all 4 receiving anti-tachycardia pacing initially and a subsequent 41J shock. All 4 patients survived post therapy with one who died due to disease progression almost 4 months later and another due to recurrent intractable VF 10 days post therapy. One patient had bradyarrhythmia and syncope and symptoms resolved on raising the lower pacing threshold. 8/15 patients had documented ventricular arrhythmias on routine ICD interrogation of whom 5/8 were established on oral antiarrhythmics.

Conclusion: This study confirms a high incidence of ventricular arrhythmias in patients with cardiac amyloidosis. Only 4 patients in this cohort received therapy from their ICD however, with two unequivocally achieving a significant survival benefit. All patients in the cohort received anti-arrhythmic therapy in addition to ICD implantation which may have reduced the incidence of serious ventricular arrhythmias. Whilst ICDs effectively treat ventricular arrhythmias in patients with cardiac amyloidosis, the survival benefit conferred by their implantation remains uncertain.


A good clonal response to chemotherapy in AL amyloidosis is associated with improved quality of life and function at 1 year

J-P Carter, DM Foard, LG Rannigan, K Aliaz, S Mahmood, S Sachchithanantham, M Fontana, C Quarta, A Martinez-Naharro, T Youngstein, T Rezk, AD Wechalekar, CJ Whelan, HJ Lachmann, PN Hawkins, JD Gillmore and T Lane
djohn-paul.carter.11@ucl.ac.uk

National Amyloidosis Centre, Division of Medicine, Royal Free Campus, University College London, UK

Background: Quality of life (QoL) is of paramount importance in chronic diseases, however, there are few data on the medium and long term QoL in patients with AL amyloidosis.

Objective: To explore changes in QoL over the disease course and treatment in AL amyloidosis.

Methods: As part of our prospective observational study of chemotherapy in AL amyloidosis (ALchemy), we examined QoL at diagnosis and 12 months (m) using the EORTC QLQ-C30. The survey incorporates functional, symptom, and global health status/QoL (GH/QoL) scales. All items range in score from 0 to 100; a high score in a functional scale and GH/QoL represents a healthy level of functioning/QoL.

Results: Baseline data from the first 500 ALchemy patients indicated significant impairment in GH/QoL and function. Patients also suffered significant fatigue, pain, dyspnoea, insomnia and gastrointestinal symptoms. 143 evaluable patients were examined at 12m. A higher proportion of patients who had a complete or very good partial response (CR/VGPR) to chemotherapy had improvement in GH/QoL than those with only a partial response (PR) or non-responders (NR): 56% CR/VGPR v 40% PR v 9% NR. In comparison to the PR group, those with CR/VGPR reported improvement in GH/QoL, SF, RF and emotional function (EF) (Figure 1). PF did not appear to improve in the CR/VGPR group and deteriorated in the PR group. Fatigue was the symptom which had improved most at 12m in the CR/VGPR group (53%), however, even amongst the CR/VGPR group ≥ 55% were still affected by gastrointestinal symptoms at 12m, and 39%, 44% and 48% of the CR/VGPR patients still reported no improvement in pain, dyspnoea and insomnia respectively.

Conclusion: Patients who achieve a good clonal response are more likely to experience improved QoL at 1 year than partial or inadequate responders. Although certain disease-related symptoms may improve in association with a good remission, others may persist, perhaps due to irreversible organ damage. Longer follow-up would be informative.

Figure 1. Change in QoL and function at 1 year. Patients who have achieved a CR/VGPR at 12 months score better than PR patients on GH/QoL, SF, RF and EF.
PB83

A clinical validation study of an amyloid fibril specific anti-lambda immunoglobulin light chain monoclonal antibody for the diagnosis of AL amyloidosis in 150 cases

JA Gilbertson, NA Botcher, P Westermark, GT Westermark, LA Smith, ER Jefferson, HJ Lachmann, JD Gillmore, C Whelan, PN Hawkins, AD Wechalekar.
National Amyloidosis Centre, Division of Medicine, University College London, London

Introduction

Accurate identification of the amyloid fibril protein is the critical first step in the diagnostic pathway for any patient with amyloidosis. Immunohistochemistry (IHC), the most widely used method to determine the fibril type, is challenging in AL amyloidosis due to background staining in formalin-fixed-paraffin-embedded-tissues (FFPE). Most laboratories (including our laboratory) use commercially available antibodies directed to the amyloid fibril proteins for immunohistochemistry. We report results of using an amyloid specific monoclonal antibody to lambda (λ) immunoglobulin light chains in 150 patients with amyloidosis compared to standard commercial polyclonal antibodies.

Method

One hundred and fifty cases (with 23 different tissue types tested) referred to the UK national amyloidosis centre with suspected AL-lambda amyloidosis were included in this study. IHC was performed on both manual and automated platforms. The commercial polyclonal antibodies to kappa and lambda immunoglobulin light chains from obtained from Dako (Dako UK Ltd, Ely, UK) and used at 1 in 20000, (manual platform) or 1 in 32000 (automated platform) dilution. The amyloid specific lambda monoclonal antibody was cell supernatant (pwlam), (supplied by PW and GW, Uppsala University, Uppsala, Sweden) used at a dilution 1/100 on an automated platform. All cases were also stained with antibodies to serum amyloid A (SAA) and transthyretin (TTR). IHC was interpreted independently by two experienced operators. Proteomic analysis (LC-MS) was performed on cases without clear IHC identification of amyloid fibril type.

Results

IHC conclusively identified the fibril protein type in 108 (72%) patients: ALκ 5 (3%) and ALλ 103 (69%). The typing was unclear by IHC in 42 (28%) cases. Staining with antibody to lambda immunoglobulin light chains using both the commercial polyclonal antibody and pwlam antibody was seen in 83 (81%) cases. Sixteen (15%) cases showed specific staining with the polyclonal anti-lambda light chain antibody only but not with pwlam antibody. While 4 (4%) stained only with the pwlam antibody and not by the commercial anti-lambda antibody. In the 42 (28%) cases without clear IHC typing, pwlam antibody did not stain any of these cases i.e. there were no false positives. Laser capture followed by mass spectrometry and proteomic analysis confirmed amyloid fibril type in 27/42 (64%) of the cases unclear by IHC (10 cases (6.6%) were inconclusive or the sample was insufficient for LC-MS) Of these cases the typing was: ALκ 6 (22%) cases, ALλ 8 (29%) cases, and a case each of lysozyme, atrial natriuretic peptide and insulin derived amyloid (4% each). The sensitivity and specificity of polyclonal antibody vs. pwlam antibody to lambda immunoglobulin light chains was 92% and 50% vs. 78% and 100% respectively giving a positive and negative predictive value for correct identification of AL-lambda type of 70% and 84% vs. 100% and 61% respectively.

Discussion and Conclusion

Immunohistochemistry gave conclusive typing in 72% of the cohort. In all positive cases, the pwlam antibody gave clean staining with no/minimal background staining and there was clean negative staining in the inconclusive cases; in contrast to the polyclonal anti-lambda antibody where significant background staining was seen. The pwlam antibody has a very high specificity but a low sensitivity for confirming the amyloid fibril type and vice versa for the polyclonal anti-lambda light chain antibody. Amyloid fibril typing by IHC remains useful and it is imperative to use a comprehensive panel of antibodies. The pwlam monoclonal antibody to lambda immunoglobulin light chains is a useful addition to the standard IHC panel and should be routinely adopted by laboratories using IHC for amyloid fibril typing. Development of a monoclonal amyloid specific antibody to kappa light chains antibody would increase the sensitivity and specificity of IHC for diagnosis of AL amyloidosis.
Characterization of altered protein expression profiles associated with light chain cardiotoxicity in human cardiac cells

F Lavatelli\textsuperscript{1}, E Imperlini\textsuperscript{2}, P Rognoni\textsuperscript{1}, M Bozzola\textsuperscript{1}, G Palladini\textsuperscript{1}, S Orrù\textsuperscript{2,3}, F Salvatore\textsuperscript{2,4}, G Merlini\textsuperscript{1,5}

\textsuperscript{1}Amyloidosis Research and Treatment Center and Department of Molecular Medicine, Fondazione IRCCS Policlinico San Matteo and University of Pavia, Pavia, Italy; \textsuperscript{2}CEINGE Advanced Biotechnologies, Naples, Italy; \textsuperscript{3}Department of Movement Sciences, Parthenope University of Naples, Italy; \textsuperscript{4}Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Naples, Italy; \textsuperscript{5}Clinical Chemistry Laboratory, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

francesca.lavatelli@unipv.it

INTRODUCTION: In AL amyloidosis, amyloid cardiomyopathy is a major prognostic determinant. Although the fibrillar deposits alter the tissue structural properties, solid evidence points to soluble amyloidogenic light chains (LC) as key mediators of proteotoxicity (1). Cellular and animal models recapitulating the features of LC proteotoxicity are the premise to define the molecular bases of damage; we previously showed that primary human cardiac fibroblasts (hCF) internalize cardiotoxic LCs, which localize to endo-lysosomes and mitochondria (2). The detailed molecular events occurring in the heart during AL amyloidosis, however, are still largely obscure. In this study, we explored the changes in the proteome of hCF incubated with exogenous cardiotoxic LCs, in order to cast light on the alterations caused by the soluble precursor.

MATERIAL & METHODS: Primary cultured hCF were incubated with LCs purified by ion exchange chromatography from patients’ urines. The LCs were classified on the basis of the clinical phenotype they determined in patients. The proteome of hCF exposed to a representative amyloidogenic cardiotoxic LC (CT-LC) was compared with that of cells exposed to a non-amyloidogenic and non-cardiotoxic LC (NT-LC) and of non-exposed cells (CTRL), using two-dimensional differential gel electrophoresis (2D DIGE). Quantitative image analysis was performed to reveal statistically significant (p value ≤ 0.05) and differentially expressed protein spots with fold changes ≥ 1.20 or ≤ -1.20; these excised spots were identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) coupled to bioinformatics, and independently validated by immunoblotting.

RESULTS: Incubation of cells with soluble CT-LCs translated into specific proteome changes that were evidenced by 2D DIGE. From all differentially expressed spots, a total of 40 were unequivocally identified as single protein species by LC-MS/MS. In particular, we identified 14 differentially expressed proteins in CT-LC vs CTRL (all underexpressed), whereas 13 differentially expressed proteins were identified in the CT-LC vs NT-LC (10 underexpressed and 3 overexpressed). Among the identified differential proteins, six were selected on the basis of their greater relative fold change and of their known biological roles, and were successfully validated by western blotting. These include two proteins related to cytoskeleton and cell shape, a mitochondrial ion channel, an intracellular chaperone and two components of the ubiquitin-proteasome system.

DISCUSSION & CONCLUSIONS: Our differential proteomics approach casts insights into the cellular and molecular changes associated with LC cardiotoxicity. These data indicate that exposure of hCF to soluble toxic LCs translates into alterations of the proteome, which may lead to functional impairment of metabolism and viability and of cytoskeleton remodelling. These features, identified in vitro, can now be assessed in human affected tissues and body fluids, as candidate biomarkers of proteotoxicity in the heart.

REFERENCES:


STABILIZATION OF ECHOCARDIOGRAPHIC INDICES OF CARDIAC STRUCTURE AND FUNCTION IN CARDIAC AL AMYLOIDOSIS FOLLOWING CLONAL RESPONSE TO CHEMOTHERAPY

CC Quarta, AD Wechalekar, D Foard, M Fontana, T. Youngstein, S. Ozer, S Sachchithanantham, S Mahmood, T Lane, JA Gilbertson, CJ Whelan, HJ Lachmann, PN Hawkins, JD Gillmore

National Amyloidosis Centre, Division of Medicine, UCL, Royal Free Hospital, London, UK
c.quarta@ucl.ac.uk

INTRODUCTION: Cardiac involvement occurs frequently in systemic light chain (AL) amyloidosis and has major prognostic implications: median survival is < 6 months in patients with markedly elevated serum cardiac biomarkers (Mayo stage 3). Whilst a prompt reduction of circulating amyloidogenic light chains through chemotherapy can substantially improve the outcome, little is known about the changes of cardiac structure and function following successful chemotherapy. We systematically analyzed echocardiography findings over time in patients with AL amyloidosis and Mayo stage 3 who achieved a sustained complete hematological response (CR) to chemotherapy.

MATERIAL & METHODS: Among the 470 consecutive patients included in the prospective ALchemy study at our Centre who were diagnosed with Mayo stage 3 AL amyloidosis between 2009 and 2014, we analyzed those who achieved a rapid and sustained CR, defined as: normalization of serum free light chain concentration and ratio coupled with absence of a monoclonal protein in serum and urine by immunofixation achieved within 6 months of diagnosis and with no relapses during follow-up.

All subjects underwent a 6-monthly protocol of evaluations including ECG, echocardiograms and laboratory tests, and we report here the findings in patients who underwent serial echocardiograms for at least 2 years. Baseline clinical, biochemical and echocardiographic findings were compared to those obtained at 12 and 24 months.

RESULTS: We identified 35 patients (age 66 [59-74] years; 57% male) with Mayo stage 3 AL amyloidosis who achieved a prompt and sustained clonal response to chemotherapy (Velcade-based chemotherapy, n=20; thalidomide-based, n=13; melphalan/dexamethasone, n=1; cyclophosphamide/dexamethasone, n=1). Twenty-nine (83%) patients had lambda light chain sub-type.

At baseline, mean left ventricular (LV) wall thickness was 15±2 mm; LV ejection fraction was 55% [53-58]; LV longitudinal strain was -11% [-13 to -8]; lateral mitral systolic velocity (S’) was 0.05 m/s [0.04-0.06]; and mitral inflow to mitral relaxation velocity ratio (E/E’) was 18 [13-23].

On longitudinal analysis, NT-proBNP fell from 4576 pg/mL [2762-7872] to 3653 pg/mL [1797-6153] at 12 months, and further to 2576 pg/mL [703-6297] at 24 months (p=0.001); LV wall thickness and LV ejection fraction did not change significantly over time (p=0.1 and p=0.87, respectively); E/E’ decreased significantly from 18 [13-23] to 16 [13-24] at 12 months to 13 [9-17] at 24 months. S’ velocity did not change during the first year, while it slightly improved to 0.06 m/s [0.05-0.07] during the second year (p<0.001); LV longitudinal strain improved from -11% [-13 to -8] to -12% [-16 to -8] at 12 months, and to -13% [-16 to -11] at 24 months (p <0.001).

DISCUSSION & CONCLUSIONS: In this systematic prospective 2 year analysis of patients with systemic AL amyloidosis and Mayo stage 3 cardiac involvement, a sustained complete response to chemotherapy was frequently associated with improvement in more recent echocardiographic parameters, such as Tissue Doppler Imaging and speckle tracking derived indices, but with no significant changes in standard 2D echocardiographic measurements, such as LV wall thickness and LV ejection fraction.

These findings highlight the role of more sensitive echocardiographic measurements such as LV longitudinal strain in tracking cardiac amyloid in clinical practice and trials of upcoming therapies.
PB86

A randomized phase III trial of melphalan and dexamethasone (MDex) versus bortezomib, melphalan and dexamethasone (BMDex) for untreated patients with AL amyloidosis

E Kastritis,1 X. Leleu,2 B Arnulfi, E Zamagni,4 MT Cibeira,5 P Kwok,6 P Mollee,7 R. Hájek,8 P Moreau,8 A Jaccard,9 S Schönländ,1 R Filshie,1 E Nicolas-Virelizier,13 B Augustson,14 MV Mateos,15 A Wechalekar,16 E Hachulla,17 P Milani,18 MA Dimopoulos,1 JP Fermand1, A Foli,18 M Gavriatopoulou,1 S Perlini,18 A Palumbo19, P Sonneveld,20 HE Johnsen,21 G Palladini

1Department of Clinical Therapeutics, National and Kapodistrian University of Athens School of Medicine, Athens, Greece, 2Hospital Huriez CHRU Lille, France, 3Hospital Saint-Louis, Paris, France, 4Bologna University School of Medicine, Bologna, Italy, 5Hospital Clinic of Barcelona, Barcelona Spain, 6Westmead Hospital, Sydney, 7Princess Alexandra Hospital and University of Queensland, Brisbane, Australia, 8University Hospital, Ostrava, Czech Republic, 9CHU Hotel Dieu, Nantes, France, 10Centre Hospitalier Universitariaire, Limoges, France, 11University Hospital, Heidelberg, Germany, 12St. Vincent’s Hospital, Melbourne, Australia, 13Centre Leon Berard, Lyon, France, 14Sir Charles Gairdner Hospital, Perth, Australia, 15University Hospital of Salamanca, Salamanca, Spain, 16University College London Medical School, Royal Free Hospital Campus, London, UK, 17Hospital Huriez, CHRU Lille, France, 18Fondazione IRCCS Policlinico San Matteo, and University of Pavia, Pavia, Italy, 19University of Torino, Torino, Italy, 20Erasmus MC Cancer Institute, Rotterdam, the Netherlands, 21Aalborg University Hospital, Aalborg, Denmark

giovanni.palladini@unipv.it

INTRODUCTION: Bortezomib is highly effective in AL amyloidosis, particularly when combined with alkylators, and these combinations are now largely used up front. However, retrospective case-control studies failed to demonstrate their superiority over standard treatments, melphalan/dexamethasone (MDex) and cyclophosphamide/thalidomide/dexamethasone. We conducted a randomized phase III trial comparing MDex and MDex plus bortezomib (BMDex) in newly-diagnosed AL amyloidosis. Enrollment is now completed (110 patients).

MATERIAL & METHODS: Patients with overt multiple myeloma, NYHA class >II, significant peripheral neuropathy, and cardiac stage III with NT-proBNP >8500 ng/L (stage IIIb) were excluded. Patients were stratified according to standard Mayo stage and randomized to receive MDex or BMDex (bortezomib added – subcutaneously starting in January 2013 – at 1.3 mg/m2, twice weekly in cycles 1 and 2 and weekly thereafter). Patients needed to have measurable disease (M-protein >10 g/L and/or dFLC >50 mg/L). The primary endpoint was overall hematologic response at 3 months. The study was supported by the European Myeloma Network, the Australasian Leukaemia and Lymphoma Group, and Janssen-Cilag.

RESULTS: Median age was 66 years and 56% of patients were male. A total of 79% and 77% of patients had cardiac amyloidosis (12% and 15% were stage Ila), and 63% and 68% had renal involvement (14% and 12% were stage IIIa) in the MDex and BMDex arms, respectively. At data lock, 99 patients (51 in the MDex arm) were evaluable for toxicity. Grade 3-4 adverse events (AEs) were more common, though not significantly, during BMDex cycles (20% vs. 14%, P=0.08), but the proportion of patients experiencing at least grade 3-4 toxicity was similar in the MDex and BMDex arms (32% vs. 34%, P=0.49). Most common grade 3-4 AEs (MDex vs. BMDex) were cytopenia (28% vs. 21%, P=0.26), fluid retention (18% vs. 10%, P=0.20), and neuropathy (0 vs. 12%, P=0.005). One patient (stage II) died within 3 months in the MDex arm and 3 (1 stage II, 2 stage IIIa) in the BMDex group (P=0.28). Ninety-two patients, 48 treated with MDex and 44 who received BMDex, were evaluable for response by intent-to-treat after cycle 3. Overall, hematologic response rates were 52% and 79% (P=0.005), with 35% and 54% CR/VGPR (P=0.05), in the MDex and BMDex arms, respectively. After a median of 5 cycles, cardiac response was reached in 6/27 (22%) and 7/23 (30%) evaluable patients (P=0.37), and renal response in 12/27 (44%) and 8/25 (32%) evaluable patients (P=0.26) with MDex and BMDex, respectively. After a median follow-up for living patients of 22 months, 75% and 85% of patients were projected to be alive at 2 years in the MDex and BMDex arms respectively (P=0.27).

DISCUSSION & CONCLUSIONS: The addition of bortezomib to MDex improves hematologic response rate with no significant increase in overall toxicity. Updated results will be presented at the meeting.
PB87

Six-minute walk test in AL amyloidosis – baseline and 12 month follow-up after chemotherapy

KED Flatman, DM Foard, E Pyart, G Hughes, R Gaudia, C Kearney, I Elmi, A Bangova, P Libo-On, J Caringal-Galima, E Rowles, C Guillotette, PN Hawkins, JD Gillmore, T Lane

kedf1@student.le.ac.uk

National Amyloidosis Centre, Division of Medicine, Royal Free Campus, University College London, UK

Background: The six-minute walk test (6MWT) provides a simple objective measure of functional exercise capacity. It is widely used in clinical practice and in clinical trials as a marker of disease severity and response to therapy.

Objective: To investigate the change in functional exercise capacity using the 6MWT in patients with AL amyloidosis who have undergone chemotherapy.

Methods: As part of our prospective observational study of chemotherapy in AL amyloidosis (ALchemy), we performed 6MWT at diagnosis (baseline) and at 12 month follow-up, after patients had received chemotherapy.

Results: 461 patients performed a baseline 6MWT between September 2012 and December 2014, and 204 were evaluable at 12 months: 106 (52%) had achieved a complete or very good partial clonal response (CR/VGPR), 77 (38%) achieved a partial response (PR) and 21 (10%) were non-responders (NR). Figure 1 shows the proportion of improvers in relation to clonal response. Of those with a CR/VGPR, 61 (58%) had improved 6MWT distance by a median of 53 m (IQR 16-19 m; mean 67 m) whilst 45 (42%) walked 56 m less than at baseline (19-98; 68). Among the PR group 30 (39%) improved by a distance of 49 m (17-97; 75) whilst 47 (61%) deteriorated by 46 m (23-119; 86). Among the NRs 5 patients (24%) improved by 84 m (53-93; 93) whilst 16 (76%) worsened by 58 m (34-138; 98).

Conclusions: A better clonal response to chemotherapy was associated with a greater proportion of patients who improved their 6MWT results at 12 months. However, a relatively high proportion of CR/VGPR patients were functionally worse at 12 months; this is consistent with patient-reported lack of improvement in physical function at 12 months, reported in a separate abstract at this meeting (Carter et al). The value and interpretation of the 6MWT in this complex heterogeneous multisystem disorder require further investigation and clarification.


Figure 1. Proportion of patients improved and worsened walking distance by clonal response at 12 months.
Correlation of cardiac biomarkers, echocardiographic findings, cardiac magnetic resonance imaging and myocardial histology in a series of patients with immunoglobulin light chain (AL) amyloidosis

MT Cibeira¹, M Solé², JT Ortiz¹, RJ Perea¹, M. Masotti³, C Fernández de Larrea¹, T Díaz¹, S. Azorín⁵, JI Aróstegui⁶, TM de Caralt⁴, X Bosch³, J Bladé¹

¹ Department of Hematology. ² Department of Pathology. ³ Department of Cardiology. ⁴ Department of Radiology. ⁵ Department of Nephrology. ⁶ Department of Immunology. Amyloidosis and Myeloma Unit, Hospital Clínic, Institut d’Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, Barcelona, Spain.

mcibeira@clinic.ub.es

INTRODUCTION: Immunoglobulin light chain (AL) amyloidosis is a plasma cell disorder characterized by extracellular deposition of monoclonal immunoglobulin light chains-derived amyloid fibrils. Kidney and heart are the most frequently involved organs, being cardiac involvement the most important prognostic factor. Endomyocardial biopsy, the gold standard for diagnosing cardiac amyloidosis (CA), is an invasive technique and is limited to experienced centers. For that reason, diagnosis in clinical practice is usually supported by echocardiographic findings, which also have some limitations, combined with a positive noncardiac biopsy. More recently, cardiac magnetic resonance (CMR) has demonstrated to be useful in identifying CA. We conducted a prospective study in order to characterize the cardiac involvement in AL amyloidosis based on clinical presentation, serum levels of cardiac biomarkers, imaging techniques and cardiac histology.

MATERIAL & METHODS: Between January 2013 and January 2016, thirty-four consecutive patients with high suspicion or biopsy-proven diagnosis of systemic AL amyloidosis at our institution were included in the study. All of them gave written informed consent. In this period of time, seven additional patients were not included in the study mainly due to poor performance status. Besides monoclonal gammopathy study, planned cardiological work-up consisted in all the following: measurement of serum cardiac biomarkers, echocardiography, cardiac magnetic resonance (CMR) with myocardial T1 mapping and extracellular volume (ECV) fraction quantification to accurately assess the degree of cardiac involvement, and endomyocardial biopsy (EMB) taken from the right ventricle (septum).

RESULTS: Thirty patients (median age 58, range 40-81; 14M/16F) were diagnosed with systemic AL amyloidosis. Light-chain isotype was lambda in 73% of them. Median serum involved free-light chain was 369 mg/L (range, 12.7-4979) and median bone marrow plasma cell infiltration was 13.5% (range, 1-53). Ten patients were classified as Mayo cardiac stage III. All patients had cardiac biomarkers and echocardiography performed. Twenty-seven patients underwent CMR and it was not feasible in 3 patients due to claustrophobia or decreased glomerular filtration rate. Cardiac histology was available in 23 patients, obtained by EMB in 22 and at cardiac autopsy in one. Among those patients with interventricular septum thickness (IVSt) of 12 mm or lower (N=11), none of them had symptoms of heart failure, none was classified as Mayo cardiac stage III, abnormal late gadolinium enhancement (LGE) was present in 78% of patients (with focal pattern in 72% of them) and amyloid deposition was demonstrated in 89% of those with available cardiac histology (with focal pattern in 50% of them). In the group of patients with suggested CA by echocardiography (IVSt over 12 mm) (N=19), symptoms of heart failure were present in 15 (79%), half of them were classified as Mayo cardiac stage III, LGE was abnormal in 89% of patients (with circumferential subendocardial or diffuse LGE pattern in most of them, 75%) and 93% had biopsy-proven CA (with diffuse pattern of deposition in 77% of them). Measurement of ECV by T1 mapping CMR technique was available in 16 patients, with values over 27% in all of them.

DISCUSSION & CONCLUSIONS: In our experience, CMR is useful to detect preclinical cardiac amyloidosis in patients with no evidence of CA by echocardiography, usually showing a focal LGE pattern and increased ECV by T1 mapping in this group of patients, also frequently associated with a focal pattern of amyloid deposition by EMB. To ascertain prognostic value of LGE patterns as well as patterns of amyloid deposition on cardiac histology, a larger series of patients and longer follow-up is needed.
Identification and quantification of urinary monoclonal proteins by capillary electrophoresis in AL amyloidosis

F Russo¹, V Valentini¹, M Basset¹, T Bosoni², P Milani¹, G Ferraro¹, L Pirolini², A Foli¹, F Lavatelli¹, F Belvisi¹, M Nuvolone¹, R Albertini², G Palladini¹, G Merlini¹,²

¹Amyloidosis Research and Treatment Center, Foundation Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, and Department of Molecular Medicine, University of Pavia, and ²Clinical Chemistry Laboratory, Foundation IRCCS Policlinico San Matteo Pavia, Italy

giovanni.palladini@unipv.it

INTRODUCTION: Identification and quantification of urinary monoclonal proteins (uMPs) is of utmost importance in the diagnosis and monitoring of monoclonal gammopathies. In AL amyloidosis, the relatively low concentration can hinder the quantification of uMPs. We prospectively assessed the performance of the Sebia Capillaries 2 Flex Piercing Urine protein capillary electrophoresis (UPCE) and immunotyping in patients with AL amyloidosis as part of a larger study involving patients with plasma cell dyscrasias.

MATERIAL & METHODS: Samples were tested with: (a) homemade high-resolution agarose gel immunofixation electrophoresis (hr-IFE) of serum and concentrated (10 times) urine; (b) commercial semi-automated agarose gel immunofixation of urine (Sebia Hydragel BJ on Hydrasys 2); (c) UPCE and immunotyping (Sebia Capillaries 2 Flex Piercing Urine); (d) quantification of circulating free light chains (FLC) by Freelite and N latex FLC. Urinary MPs were quantified using Sebia Phoresis software tools. Only patients in whom uMPs were detected by hr-IFE were included in the study.

RESULTS: 68 patients with AL amyloidosis were included. The heart was involved in 41 patients (60%) and the kidney in 36 (53%). Estimated glomerular filtration rate was <30 mL/min in 9 (13%) and <15 mL/min in 4 (6%) cases. Table 1 shows the diagnostic sensitivity of the tests. A uMP was detected by UPCE in 62 patients (91%), and was quantifiable in 55 cases (81%). The median uMP excretion was 130 mg/24h (range 10-1610 mg/24h) as assessed by Phoresis tool. Interestingly, 9 of the 12 patients with non-measurable dFLC (Freelite) (<50 mg/L) had a quantifiable uMP (median 90 mg/24h). The uMP was also quantifiable on Hydragel BJ agarose gel in 51 patients (75%). There was a good correlation between measurements of uMP excretion on Capillaries and Hydragel (Pearson’s r = 0.87, 95%CI 0.78-0.92). So far, 16 patients with quantifiable uMP and dFLC (Freelite) >50 mg/L were treated and had response data at 3 months. Five subjects responded (1 PR, 4 VGPR) with a median 69% dFLC decrease (range 51-90%). In all of them uMP excretion decreased (median 100%, range 30-100%). Among non-responders, only one patient had a relevant reduction in uMP excretion (from 740 to 250 mg/24h, dFLC from 746 to 619 mg/L) with stable renal function. Post-treatment UPCE was also available in 5 patients with baseline dFLC (Freelite) <50 mg/L. In 2 of them the uMP was still visible but was no longer quantifiable, in 2 it remained stable and in one patient uMP increased from 20 to 40 mg/24h.

DISCUSSION & CONCLUSIONS: Capillaries 2 Flex Piercing Urine protein electrophoresis can identify uMPs in patients with AL amyloidosis with a good sensitivity, and can quantify uMP excretion as low as 10 mg/24h. Changes in uMP excretion can be monitored during treatment, including some patients without dFLC-measurable disease. Further studies are warranted to evaluate this tool in response assessment.

Table 1. Diagnostic sensitivity in 75 patients with a uMP detectable at hr-IFE of concentrated urine

<table>
<thead>
<tr>
<th>Assay</th>
<th>N (%)</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>hr-IFE of serum</td>
<td>68 (100)</td>
<td>96-100</td>
</tr>
<tr>
<td>Freelite ratio</td>
<td>58 (85)</td>
<td>75-92</td>
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<tr>
<td>N latex FLC ratio</td>
<td>57 (84)</td>
<td>74-91</td>
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<td>semi-automated IFE of urine</td>
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<td>UPCE</td>
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CHEMOTHERAPY BEFORE AND AFTER HEART TRANSPLANTATION FOR PATIENTS WITH ADVANCED CARDIAC AL AMYLOIDOSIS, SINGLE CENTER RESULTS WITH LONG-TERM FOLLOW-UP


1 Amyloidosis Center, Dept. of Hematology, Oncology and Rheumatology, University of Heidelberg, Heidelberg, Germany, 2 Department of Cardiology, University of Heidelberg, Heidelberg, Germany, 3 Dept. of Cardiac Surgery, University of Heidelberg, Heidelberg, Germany

Katharina.haack@med.uni-heidelberg.de

INTRODUCTION: Survival rates for patients with light-chain (AL) amyloidosis are gravely reduced by advanced cardiac involvement at Mayo stage III with a median survival of 3.5 months [1]. High-dose Melphalan (HDM) and autologous stem cell transplantation (ASCT) or other intense chemotherapy regimen cannot be applied to those patients, as it comes with great risk of therapy-related mortality [2]. However, due to the systemic nature of the disease, some medical centers decline heart transplantation as treatment in AL [3]. Our aim was to examine the cases of cardiac AL patients treated with heart transplantation (HTx), combined with chemotherapy and/or HDM/ASCT in our institution and re-evaluate the necessity and clinical outcome of this treatment approach.

PATIENTS & METHODS: Data from 38 patients (19m, 19f) suffering from cardiac AL who were treated in our hospital between 2002 and 2015 were retrospectively analyzed. All patients were high-urgency listed for orthotopic HTx due to poor perspective of survival. Until 2009, 13 patients were listed, 8 of them with multiple organ involvement. Thereafter, we excluded patients with multiple organ involvement. All data are derived as medians with range or absolute numbers. Survival curves were calculated using the Kaplan-Meier method.

RESULTS: Median age was 50 years (35-62) at diagnosis. Amyloidogenic lambda light-chains (LC) were detected in 32 and kappa LC in 6 patients. Median dFLC was 564,0 (68,9 – 2752,0) and median plasma cells in bone marrow were 14% (5-35). Median NT-proBNP was 6780 ng/l (1500 -53194), median cTNT 0,11 µg/l (0,01-0,52) and median hsTNT was 94 ng/l (28-448) at diagnosis. Median NYHA stage was 3 (2-3) and median MAYO 2004 stage was 3 (2-3). Serum creatinine was at a median of 0,94 mg/dl (0,66-2,45), proteinuria at 0,1 g/day (range 0-10,7). Patients stayed on the high-urgency waiting list for a median of 26 (range 3-54) before 2009, and a median of 63 days (15-259) after 2009. 33 patients were treated with chemotherapy prior to HTx (mostly dexa w/o Bortezomib) to reduce dFLC during the waiting time. With a median of 5 months after HTx (4-29), 18 patients received ASCT. HDM was used with either 200 mg/m2 (N=10) or reduced dosage (N=8) in patients with reduced kidney function (mostly due to renal complications after HTx). Complete remission (CR) was achieved in 7 patients, (24% of all transplanted pts, n=29; 2 patients have not finished treatment yet.) very good partial remission (VGPR) in 6 patients in 6 (21%) and partial remission (PR) in 7 patients (24%). Overall, 24 patients died. Cause of death was either progression of AL (N=15), sepsis (n=4), heart transplant rejection (n=3) or other (n=2). 7 patients died before receiving HTx with a median survival (start point: HU listing) of 16 days (6-61). Patients that underwent HTx had a median survival of 46 months (3-175, 1-year survival: 77%).

DISCUSSION & CONCLUSION: HTx followed by chemotherapy is a feasible treatment approach in patients with advanced cardiac amyloidosis. Patients who reach HTx have a nearly 50% chance for a good hematologic remission (VGPR or better) and consecutively a favorable survival probability with a median OS of nearly 4 years in our series.

INTRODUCTION: Interphase fluorescence in situ hybridization (iFISH) of plasma cells has become a diagnostic standard for risk stratification in multiple myeloma. However, in AL amyloidosis (AL), a related plasma cell dyscrasia, the prognostic impact of cytogenetic aberrations is still controversial. Therefore, it was the aim of this long term follow-up study to analyse the prognosis of AL patients (pts.) treated with high dose melphalan chemotherapy (HDM) depending on iFISH results.

MATERIAL & METHODS: We analysed a consecutive cohort of 123 AL pts. from 02/2003 to 05/2014. Pts with age up to 70 years, NYHA stage ≤ 2 and ECOG ≤ 2 were considered for HDM. Median age was 55 years (37-70), median dFLC 143.4 mg/l (0 – 3194), median number of involved organs 2 (1-5), 70/123 (57%) had cardiac and 82/123 (67%) had renal involvement. 10/123 (8%) had received heart transplantation prior to HDM. 70/123 pts. (57%) received an induction therapy - among them 32 a bortezomib based regimen - and 101/123 (82%) a mobilisation chemotherapy prior to stem cell harvest. High dose therapy was administered with melphalan 2x100 mg/m² with respective adjustments for renal function. iFISH cytogenetics was performed in plasma cells purified by auto-magnetic-activated cell sorting with CD138 immunobeads. For hybridization commercial two-color probe sets were used.

RESULTS: Expectedly, translocation t(11;14) was the most prevalent aberration (59%), followed by deletion 13q14 (29%), gain 1q21 (22%), hyperdiploidy (14%) and the high risk aberrations t(4;14), t(14;16) or deletion 17p13 (7%). Detection of t(11;14) conferred a favourable prognosis with superior complete remission rates (41.2% vs. 20.0%, p=0.02) and hematologic event free survival (hemEFS) (median 46.1 vs. 28.1 months, p = 0.05). Statistical significance was not reached for overall survival (OS) (5-year OS 78.8% vs. 67.3%, p = 0.07). The favourable prognostic effect of t(11;14) regarding hemEFS was confirmed as an independent factor (p = 0.008) in a multivariate model incorporating age, light chain restriction, dFLC, Mayo score, MDRD and melphalan dosage, along with higher dFLC as the only other significant risk factor (p = 0.002). High risk aberrations conferred inferior complete remission rates (0%, p = 0.03), inferior hemEFS (28.1 vs. 32.4 months, p=0.30) and OS (5-year OS 33.3% vs. 76.5%, p=0.06). Gain 1q21 had a trend for a worse hemEFS (31.1 vs. 44.0 months, p=0.13), which did not translate into poorer OS (p=0.93). Deletion 13q14 and hyperdiploidy were prognostically neutral as well.

DISCUSSION & CONCLUSIONS: This study confirms the efficacy of HDM for eligible pts.. In this long term follow-up study, we could show that t(11;14) positive pts. benefit in particular from HDM and are those most likely to achieve long lasting remission and survival. Given the adverse prognosis of t(11;14) positive patients in the setting of bortezomib therapy, this highlights the impact of administered therapy on the prognostic role of iFISH.

OUTCOMES OF PATIENTS WITH CARDIAC AMYLOIDOSIS AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION AND ITS ASSOCIATION WITH LONGITUDINAL STRAIN ANALYSIS PER SPECKLED TRACKING ECHOCARDIOGRAPHY

RA Quintana-Quezada¹, J Banchs¹, SW Yusuf¹, MH Qazilbash²

¹ Department of Cardiology, MD Anderson Cancer Center, USA. ²Stem Cell Transplantation and Cellular Therapy, MD Anderson Cancer Center, USA.

mqazilba@mdanderson.org

Background: Cardiac involvement (CA) is a predictor of unfavorable outcome in light-chain amyloidosis (AL).[1] Although high-dose therapy and autologous hematopoietic stem cell transplantation (auto-HCT) is an effective therapy for AL, concurrent CA is associated with higher transplant-related mortality (TRM). Cardiac mechanical deformation measures are better predictors of outcome than left ventricular (LV) ejection fraction.[2] CA is a continuum that starts in the subendocardium of the base, sparing the apex.[3,4] We evaluated the role of longitudinal strain (LS) per speckled tracking echocardiography (STE) and report its impact on the outcome.

Methods: From 1998 to 2014, a total of 37 patients with AL and CA underwent auto-HCT at our institution. Using STE, LS values from basal, mid and apical segments of the LV were averaged into 3 regional values. Organ involvement and responses were defined according to established consensus criteria.

Results: Median age at transplantation was 56 years. Concomitant other organ involvement was: kidney in 17(46%), GI in 11(30%), neurologic in 4(11%), liver in 2(5%) and lung in 2(5%) patients. At 6-month post-auto-HCT, of the 31 patients evaluable for response, 7(22%) had cardiac response, 15(48%) had stable disease and 8(25%) had progression. Pre- and post-transplant LS values are summarized in Table 1. Post-transplant LS improvement was mostly at the mid-segment of the LV, which did not reach statistical significance (p=0.06). The 1-year-TRM was 5.4%. At 1 year, overall organ response was observed in 15(48%) patients.

Conclusions: Our study showed that auto-HCT is safe in patients with AL and CA. Also, we found the largest LS improvement in the mid-region of the heart post-transplant.

Table 1. LS values before and after auto-HCT

<table>
<thead>
<tr>
<th></th>
<th>Pre-Transplant</th>
<th>Post-Transplant</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global (Median)</td>
<td>11.26</td>
<td>11.21</td>
<td>0.10</td>
</tr>
<tr>
<td>Apex (Median)</td>
<td>13.4</td>
<td>13.67</td>
<td>0.07</td>
</tr>
<tr>
<td>Mid (Median)</td>
<td>10.51</td>
<td>11.13</td>
<td>0.06</td>
</tr>
<tr>
<td>Basal (Median)</td>
<td>9.06</td>
<td>8.15</td>
<td>0.49</td>
</tr>
</tbody>
</table>

References:

Lenalidomide/Melphalan/Dexamethasone in newly diagnosed patients with advanced AL Amyloidosis: results of a phase 2 study with long-term follow-up

Ute Hegenbart1, Tilmann Bochtler1, Axel Benner2, Natalia Becker6, Christoph Kimmich1, Arnt V. Kristen3, Hartmut Goldschmidt1, Dirk Hose1, Anna Jauch4, Anthony D. Ho1 and Stefan Schönland1

1Amyloidosis Center, Dept. of Hematology, Oncology and Rheumatology, University of Heidelberg, Heidelberg, Germany, 2Division of Biostatistics, German Cancer Research Center, Im Neuenheimer Feld 280, 3Department of Cardiology, University of Heidelberg, 4Institute of Human Genetics Heidelberg, Germany

Ute.hegenbart@med.uni-heidelberg.de

INTRODUCTION: Chemotherapy in light chain (AL) amyloidosis aims to normalize the involved free light chain in serum, which leads to an improvement or at least stabilization of organ function in most responding patients

PATIENTS & METHODS: We performed a prospective single center phase 2 trial with 50 untreated patients not eligible for high-dose treatment. The treatment schedule comprised 6 cycles of oral lenalidomide, melphalan and dexamethasone every 4 weeks (L-M-dex). This study is registered at www.clinicaltrials.gov as #NCT00883623.

RESULTS: Complete remission (CR, primary endpoint) was achieved in 20% of 45 evaluable patients receiving at least three cycles. Hematologic and cardiac toxicities were predominant adverse events (AE; 16 serious AE, 19 AE > grade 4, two treatment related deaths). However, the early death rate was low with 4% despite inclusion of 36% of patients with cardiac stage Mayo 3. As secondary endpoint outcome was compared with a historical group of 49 AL patients who received M-Dex and were matched for the inclusion criteria. After a median follow-up of 50 months for L-M-dex and 87 months for M-Dex group, overall survival was significantly improved using L-M-dex (median 67.5 versus 26.2 months, p=0.02, figure). There was also a higher CR plus very good partial remission rate (n=25/50, 50% versus n=12/49, 24%, p=0.01), and a better event-free-survival in the L-M-dex group (median 25.1 versus 16.1 months, p=0.005).

DISCUSSION & CONCLUSION: The combination treatment of L-M-dex is effective and feasible in patients who are not eligible for high-dose treatment. L-M-dex is a promising upfront treatment for AL patients with polyneuropathy; a further advantage is the oral availability. However, a rigid surveillance is needed to reduce toxicity and mortality. Therapy results seem to be superior to M-Dex 40 mg and might be further improved using dex once per week and lenalidomide maintenance in patients with hematological remission and a good tolerability of this treatment.

Figure
PB94

EGCG rescues fibrillogenesis of light chains from patients with AL amyloidosis

Kathrin Andrich1,3, Ute Hegenbart2, Stefan Schönland2, Erich Wanker1, and Jan Bieschke3

1. Max-Delbrück-Centrum for Molecular Medicine, Department of Neuroproteomics, Berlin, 13125, Germany. 2. University Hospital Heidelberg, Department of Internal Medicine V (Hematology / Amyloidosis Center-) Heidelberg, 69120, Germany. 3. Washington University in St. Louis, Department of Biomedical Engineering, St. Louis, MO 63130

Bieschke@wustl.edu

INTRODUCTION: In both systemic light chain amyloidosis (AL) and Multiple Myeloma (MM) large amounts of soluble immunoglobulin light chains (LC) are secreted but only in AL they are forming amyloid deposits in vivo. Hence we hypothesize that amyloid deposition depends on the amyloid formation propensity of the individual LC sequences rather than being a result of different LC concentrations, which may alter susceptibilities to the green tea phenol Epigallocatechin-3-gallate (EGCG). This project is part of GERAMY (German Consortium of AL Amyloidosis).

MATERIALS & METHODS: To test this hypothesis we used a simple dialfiltration approach to isolate nine LC (2 kappa-AL; 2 lambda-AL; 2 kappa-MM; 3 lambda-MM) from the urine of patients. We quantified the thermodynamic stabilities of the individual LC proteins and monitored their aggregation under physiological conditions by Thioflavin T (ThT) fluorescence, light scattering and SDS-stability. Aggregated LC were imaged by atomic force microscopy.

RESULTS: We found that LC isolated from urine existed as dimers, 30-50% of which were crosslinked by disulfide bridges. LC formed amyloid only under reducing conditions, suggesting that monomer formation is a necessary step in LC amyloid formation. LC proteins from AL and MM patients had similar thermodynamic stabilities and formed amyloid fibrils at comparable concentrations, but individual LC featured highly diverging aggregation kinetics. Aggregation kinetics displayed two distinct phases, which corresponded to the formation of oligomers and amyloid fibrils, respectively. EGCG specifically inhibited the second aggregation phase and induced the formation of SDS-stable, non-amyloid LC aggregates.

DISCUSSION & CONCLUSION: Systematic differences in amyloid formation exist between MM and AL-LC aggregation. Aggregation kinetics were characteristic for individual patient LC samples and did not correlate with thermodynamic stabilities of LC proteins. EGCG inhibits amyloid formation following a common mechanism between LC and other amyloidogenic proteins.
Evaluation of the chromosomal aberration pattern in immunoglobulin light chain amyloidosis


1Department of Medicine, 2Department of Pathology, 3Department of Laboratory Medicine & Genetics, 4Department of of Neulorogy, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea.

redmin07@gmail.com

//INTRODUCTION:// Immunoglobulin light chain (AL) amyloidosis is a rare clonal plasma cell disorder which leads to multi-organ dysfunction by deposition of circulating amyloid fibrils. Chromosomal aberrations of plasma cells are well recognized as important pathogenetic and prognostic factors in multiple myeloma, whereas it has remained unclear in AL amyloidosis in particularly Asian patients. The purpose of this study is to identify prognostic cytogenetic risk factors by interphase fluorescence in situ hybridization (FISH) in AL amyloidosis cohort patients in Korea. //MATERIAL & METHODS:// A total of 204 patients with systemic AL amyloidosis were retrospectively analyzed from April 1995 to September 2015 in our institution. The diagnosis of AL amyloidosis required tissue confirmation of amyloid deposits or fibrils by apple-green birefringence with Congo red staining and kappa or lambda restriction by immunohistochemistry in at least one involved organ. //RESULTS:// Cytogenetic testing by FISH had been performed in 99 (49%) patients with AL amyloidosis, and 35 (35%) patients had abnormal FISH as follows; most common abnormality is gain of 1q21 (17%) followed by t(11;14), t(4;14), deletion 13, deletion 17, t(14;20) and t(14;16). Patients with abnormal FISH results had more bone marrow plasma cells (median 13.5 vs 20.1 %, p = 0.034). Median overall survival (OS) was significantly longer in patients without gain of 1q21 (50.8 months) as compared to in patients with gain of 1q21 (3.6 months, p = 0.016). There was no statistical significantly difference, but patients with t(11;14) or deletion 13 had a shorter median OS (9.9 versus 27.8 months, p = 0.334 or 3.0 vs 24.9 months, p = 0.300). //DISCUSSION & CONCLUSIONS:// In conclusion, our results suggest that FISH test is important in patients with AL amyloidosis and that detection of gain of 1q21 is an adverse prognostic factor in these patients. And further prospective and large studies of FISH test are warranted.


Table1. Frequencies of chromosomal aberrations

<table>
<thead>
<tr>
<th>No. of patients /performed FISH</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH performed</td>
<td>99</td>
</tr>
<tr>
<td>FISH abnormality</td>
<td>35</td>
</tr>
<tr>
<td>1q21 (tri1q)</td>
<td>15/90</td>
</tr>
<tr>
<td>t(11;14)</td>
<td>10/73</td>
</tr>
<tr>
<td>t(4;14)</td>
<td>9/93</td>
</tr>
<tr>
<td>del 13 (13q14 or 13q34)</td>
<td>8/97</td>
</tr>
<tr>
<td>del 17 (17p13)</td>
<td>6/94</td>
</tr>
<tr>
<td>t(14;20)</td>
<td>2/32</td>
</tr>
<tr>
<td>t(14;16)</td>
<td>5/91</td>
</tr>
</tbody>
</table>
Changes in health-related quality of life following treatment in patients with AL amyloidosis

V Sanchorawala, S Lo, MK White, KL McCausland, M Bayliss, S Guthrie, M Skinner

Amyloidosis Center, Boston University School of Medicine, Boston, Massachusetts. Optum, Lincoln, Rhode Island. Prothena Biosciences Inc, South San Francisco, United States

INTRODUCTION: Previous research has reported improvements in health-related quality of life (HRQoL) following high-dose melphalan and stem cell transplantation (SCT) in AL amyloidosis patients. Although pre-treatment deficits in HRQoL have been observed among patients deemed ineligible for SCT, little is known about the impact of non-SCT treatments on HRQoL in this disease population. Furthermore, standard non-SCT chemotherapy treatment regimens are generally associated with poor tolerability and treatment burden which may have subsequent implications on post-treatment levels of HRQoL. The purpose of this analysis is to examine patterns of HRQoL among patients with AL amyloidosis who received SCT and patients who received standard chemotherapy regimens without SCT.

METHODS: Patients with AL amyloidosis evaluated at the Amyloidosis Center at Boston University between 1994 and 2014 were asked to complete the SF-36v1 Health Survey during clinic visits. The SF-36v1 assessed HRQoL across eight domains and two component summary measures: physical functioning (PF); role limitations due to physical health problems (RP); bodily pain (BP); general health (GH); vitality (VT); social functioning (SF); role limitations due to emotional health problems (RE); mental health (MH) and physical (PCS) and mental (MCS) component summaries. SF-36v1 forms completed within 90 days of initiating treatment were used to quantify pre-treatment baseline levels of HRQoL. Post-treatment assessments of HRQoL were collected between 6 and 18 months follow-up. Complete case analytic samples, where baseline and follow-up SF-36v1 data were available for each patient, were created for each treatment group (SCT: n=188; non-SCT chemotherapy regimens: n=42). Baseline characteristics of patients in each treatment group were compared using two-sample t-tests and chi-square tests for continuous and categorical variables, respectively. Analysis of variance was used to compare the norm-based SF-36v1 scores for each treatment group at baseline and follow-up to a general U.S. population (USP). Regression models using each SF-36v1 domain or summary score as a dependent variable were used to adjust the USP to the age and gender distribution of each treatment group. Paired t-tests were conducted to test for significant differences between baseline and follow-up HRQoL within each treatment group. To examine the role of treatment on change in HRQoL, repeated measures analyses based on all SF-36 observations were conducted using generalized estimating equation models which further controlled for age, gender, and cardiac involvement.

RESULTS: Patients treated with SCT differed significantly from patients treated with non-SCT chemotherapy regimens by age, gender, types of organ involvement, and unadjusted baseline PF, VT, and PCS scores. Diminished pre-treatment HRQoL was prevalent in both groups relative to the USP. Paired t-tests supported significant improvements in HRQoL, as measured by PF, RP, VT, SF, RE, MH, and MCS scores, among patients who received SCT (p < 0.01 for all). While patients who received SCT continued to report significant deficits at follow-up for PF, RP, GH, SF, and PCS, improvements in VT, RE, MH, and MCS led to comparable scores to the USP. No significant improvements in HRQoL scores were observed among patients receiving non-SCT chemotherapy regimens over time based on paired t-tests; however, a significant reduction in GH occurred among these patients following treatment (40.0 vs 35.7, p<0.01). GEE models further supported consistent improvements across health domains among SCT patients; a significant decline in GH among non-SCT chemotherapy patients; and variation in the magnitude of observed changes by treatment group.

DISCUSSION & CONCLUSIONS: On average, patients exhibited significant improvements in many aspects of HRQoL following a SCT. In contrast, non-SCT chemotherapy patients reported a significant decline in general health following treatment. Given potential differences in disease prognosis and characteristics at baseline, inferences across treatment groups should be made with caution; however, repeated measures analyses controlling for age, gender, and cardiac involvement further supported these patterns. There is a significant need for safe and effective treatments and interventions that improve HRQoL for patients who are not eligible to receive SCT.

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The incidence of atrial fibrillation among patients with AL amyloidosis undergoing high dose melphalan and stem cell transplantation: experience at a single institution.

M Arun1, D Brauneis2,4, FL Ruberg3,4, AC Shelton3,4, JM Sloan2,4, K Quillen2,4, V Sanchorawala2,4, C Varga2,4

1 Department of Internal Medicine, Boston Medical Center, Boston, MA. 2 Department of Hematology & Medical Oncology, Boston Medical Center, Boston, MA. 3 Section of Cardiovascular Medicine, Department of Medicine, Boston Medical Center, Boston, MA. 4 Amyloidosis Center, Boston University School of Medicine, Boston, MA. Monica.Arun@bmc.org

INTRODUCTION: High dose melphalan and autologous stem cell transplantation (HDM/SCT) can induce hematologic responses and prolong survival in selected patients with AL amyloidosis. Cardiac toxicity associated with HDM/SCT remains an ongoing concern in patients with AL amyloidosis. Atrial fibrillation (AF) may complicate SCT 4-10% of the time. We sought to determine the incidence of AF in patients with AL amyloidosis undergoing SCT at Boston Medical Center.

MATERIAL & METHODS: We retrospectively analyzed the charts of 91 consecutive patients with AL amyloidosis undergoing HDM/SCT between January 2011 and May 2015. The peri-transplant period was defined as the first day of stem cell mobilization until the time of neutrophil engraftment. Medical records were reviewed for age, gender, history of AF, baseline troponin I and BNP, baseline echocardiography, dose of melphalan, ventricular rate, hemodynamic stability, AF management, and the return to normal sinus rhythm (NSR). We compared baseline characteristics between patients who developed AF in the peri-transplant period and patients who did not.

RESULTS: Ninety-one patients with AL Amyloidosis underwent HDM/SCT from January 2011 to May 2015. Overall, twelve patients (13.1%) developed AF during the peri-transplant period, at a median of D+9 (range: D-10 to D+21). Mean age was 58.3 years (range: 40-68). Among these patients, seven (58.3%) had documented cardiac involvement by amyloidosis at baseline. Four patients (33.3%) had a history of PAF, all of whom were on rate control medications at the time of SCT. Eleven patients (91.6%) had documented rapid ventricular rates (range: 103-159 beats per minute), seven (58.3%) of which were hypotensive (systolic BP < 105) at the time. Eight patients (66.7%) were treated with beta-blockers, three patients received amiodarone in addition to a beta blocker, while one patient was continued on his home dose of beta blocker. All twelve patients spontaneously converted back to NSR (range: <24 hours to > 1 week from onset), although four patients experienced recurrent episodes throughout transplant. One patient permanently converted into AF in the post-transplant period. There were no significant differences in the baseline characteristics of the patients who developed AF and the patients who did not (See Table 1). Interestingly, there were three patients who had a history of PAF but did not develop AF during the peri-transplant period.

DISCUSSION: AF occurred in 13.1% of patients with AL amyloidosis undergoing HDM/SCT, a rate higher than reported in other patient populations. Hemodynamic instability was commonly observed. Of note, 42% of patients did not have known cardiac disease at baseline, while 67% of patients had no history of AF prior to SCT. Of the 7 patients with a history of AF, only 4 had recurrences during their SCT. None of the baseline characteristics were found to be significant as risk factors for developing AF.


Table 1. Baseline cardiac characteristics

<table>
<thead>
<tr>
<th></th>
<th>Patients who developed AF</th>
<th>Patients who did not develop AF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Modified Cardiac Stage, (range)</td>
<td>II (I-III)</td>
<td>II (II-III)</td>
<td>0.35</td>
</tr>
<tr>
<td>Mean BNP, pg/mL (range)</td>
<td>200 (17-558)</td>
<td>206 (4-1015)</td>
<td>0.92</td>
</tr>
<tr>
<td>Mean Troponin, ng/mL (range)</td>
<td>0.099 (0.006 – 0.446)</td>
<td>0.053 (0.006-0.484)</td>
<td>0.30</td>
</tr>
<tr>
<td>Mean IVSD, mm (range)</td>
<td>12.0 (8-17)</td>
<td>11.3 (6-21)</td>
<td>0.40</td>
</tr>
<tr>
<td>Mean Left atrial size, mm (range)</td>
<td>34.4 (23-43)</td>
<td>33.4 (24-48)</td>
<td>0.63</td>
</tr>
</tbody>
</table>
Clinical experience with daratumumab in AL amyloidosis and MM with AL amyloidosis

BM Weiss, A Waxman, AD Cohen, LM Dember, J Zonder

Penn Amyloidosis Program, Division of Hematology-Oncology, Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA, USA; Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA

brendan.weiss@uphs.upenn.edu

INTRODUCTION: Daratumumab (DARA) is a monoclonal antibody targeting CD38 that was recently approved for relapsed and refractory multiple myeloma (MM) based on overall response rate of 29% and progression free survival of 3.7 months. Patients with AL amyloidosis (AL) often fail to achieve adequate hematologic responses despite multiple regimens. Patients with relapsed MM can develop significant organ dysfunction from amyloidosis. DARA requires prolonged high volume infusions and is associated with mild to moderate infusion-related reactions (IRRs) in nearly half of patients, both of which represent challenges in patients with multisystem organ dysfunction from AL. We present four patients with AL or MM/AL who are being treated with DARA.

MATERIALS & METHODS: Four patients with AL or MM/AL with hematologic and/or organ progression were treated with DARA. Patients 01-03 received montelukast on the day prior, the day of and two days after DARA. Patients with comorbid pulmonary disease received inhaled corticosteroids. Patients 01 and 02 received the following modification from the standard DARA treatment: DARA 8 mg/kg in 500 mL on cycle 1, day 1, then 16 mg/kg in 500 mL on day 8 and henceforth. Patients received standard medications pre- and post-DARA. Hematologic response was assessed by Consensus Criteria.

RESULTS: The details of the four patients are in the table below. All patients were heavily pretreated with at least 3 prior lines of therapy, including high-dose melphalan and autologous stem cell transplantation in all patients. Patient 01 had relapsed and refractory lambda-light chain MM with deletion 17 and developed symptomatic heart failure and multisystem amyloidosis. Patient 01 had a significant IRR (rigors), but otherwise there were no severe IRRs, other adverse events, decompensation of heart failure or worsening edema. In all 3 patients with measurable disease, there was a hematologic response. Updated data, including additional patients, will be presented at the meeting.

DISCUSSION & CONCLUSIONS: This limited experience suggests that DARA can be administered safely in patients with AL amyloidosis with cardiac and renal involvement. Hematologic responses are encouraging. Updated data, including additional patients, will be presented at the meeting.


<table>
<thead>
<tr>
<th>ID</th>
<th>Diagnosis</th>
<th>Organ involvement</th>
<th>Age/Gender</th>
<th>No. Prior regimens</th>
<th>Infusion time C1D1</th>
<th>Best hematologic response</th>
<th>NT-ProBNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>MM/AL</td>
<td>Cardiac, carpal tunnel, macroglossia</td>
<td>59/female</td>
<td>6</td>
<td>6 h, 44 min</td>
<td>CR</td>
<td>C1 2275 C2 3757</td>
</tr>
<tr>
<td>02</td>
<td>AL</td>
<td>Cardiac, bone, soft tissue</td>
<td>71/male</td>
<td>4</td>
<td>5 h, 46 min</td>
<td>PR</td>
<td>C1 7325 C2 3961</td>
</tr>
<tr>
<td>03</td>
<td>AL</td>
<td>Renal, Stage III</td>
<td>70/male</td>
<td>4</td>
<td>8 h, 27 min</td>
<td>Unmeasurable</td>
<td>NA</td>
</tr>
<tr>
<td>04</td>
<td>MM/AL</td>
<td>GI</td>
<td>56/female</td>
<td>3</td>
<td>5 h, 0 min</td>
<td>VGPR</td>
<td>NA</td>
</tr>
</tbody>
</table>

MM – multiple myeloma, AL – light chain amyloidosis, C1D1 = cycle 1, day 1; CR – complete remission, PR – partial remission, NA – not applicable
Cytogenetic abnormalities in patients with newly diagnosed AL amyloidosis

H Yameen¹, JM Sloan¹, C Varga¹, JC Lee², CJ O’Hara², V Sanchorawala¹

¹ Amyloidosis Center, Boston University School of Medicine, Boston, MA, USA. ² Department of Pathology and Laboratory Medicine, Boston Medical Center, Boston, MA, USA.

Hassan.Yameen@bmc.org

INTRODUCTION: While interphase fluorescent in situ hybridization (iFISH) is used frequently to detect chromosomal aberrations (CA) in multiple myeloma (MM) which carry prognostic implications, relatively little is known about their frequencies and prognostic implications in AL amyloidosis. Recent retrospective studies have shown that certain CAs like t(11:14)¹⁻², deletion of 13q¹⁻², and gain of 1q¹¹ are frequently seen in these patients. A recent study also showed that Bortezomib may be associated with adverse outcomes in patients with AL amyloidosis carrying t(11:14)³.

MATERIAL AND METHODS: Using our database, we reviewed 138 patients with newly diagnosed AL amyloidosis between January 2013 and June 2015. We looked at iFISH results performed on a bone marrow aspirate as part of their initial evaluation. iFISH was performed at Quest Diagnostics. For a few patients, they already had iFISH data performed prior to their initial visit with us. Quest Diagnostics uses a bispecific tetrameric antibody complex (TAC) that recognizes both CD138 and dextran. Dextran coated magnetic particles are added which binds to the TAC and the target cells (which are bound to the TAC and magnetic particles) are then separated from unlabelled cells by placing the cell suspension in a magnetic field. Nine different FISH probes for common aberrations in MM are then added and hybridized overnight. For patients with t(11:14), we also looked at the treatment they received, and the subsequent hematologic response.

RESULTS: Of 138 patients, no CAs were detected in 45.7% (n=63). IgH rearrangement was detected in 38.4% (n=53) with t(11:14) accounting for 6% (n=8/138). IgH rearrangement with an undetermined partner chromosome was seen in 29.7% (n=41/138). Deletion of 13q accounted for 24.6% (n=34) and gain of 1q accounted for 2.9% (n=4) of patients. Among the 8 patients with t(11:14), 5 were treated with a Bortezomib based regimen, and 3 with high dose melphalan and stem cell transplantation (HDM/SCT), leading to a hematologic response of 40% in Bortezomib treated patients and 66.6% in HDM/SCT treated patients.

DISCUSSION AND CONCLUSIONS: Unlike recent retrospective studies that have reported a high frequency of CAs like t(11:14), deletion of 13q, and gain of 1q in patients with AL amyloidosis, our data suggests that these CAs are detected in lower frequencies. Almost half of our cohort showed no CAs on iFISH analysis while IgH rearrangements (with any partner chromosome) were found in just over a third of our patients, deletion of 13q present in a fourth of our patients and gain of 1q detected in very few patients. The discrepancy in results could possibly be related to technical issues such as dilution of aspirate samples or inability to test all probes due to samples being insufficient in quantity. While our pool of t(11:14) patients is very small, it is notable that most patients who received HDM/SCT had at least a VGPR or better while most patients who received a Bortezomib based regimen had no response, which is in line with what is reported in the literature.


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Risk-adapted melphalan and stem cell transplantation in patients with systemic AL amyloidosis (AL) and suboptimal responses to bortezomib-based initial therapy

SW Wong1,2, D Larive3, M Warner1, KA Sprague1,2, T Fogaren1,2, RL Comenzo1,2
1The John C Davis Myeloma and Amyloid Program, Tufts Medical Center, Boston, MA, USA. 2Division of Hematology-Oncology, Tufts Medical Center, Boston, MA, USA.

The treatment of newly diagnosed patients with AL has dramatically improved with the use of the first-in-class proteasome inhibitor bortezomib in initial therapy. Overall hematologic response rates of 62% have been reported in a large series with 43% of patients achieving very good partial (VGPR) or complete responses (CR), i.e. > VGPR. In many cases, however, patients have suboptimal hematologic responses such as partial or no response (PR, NR) and are therefore likely to have inferior overall survival. Risk-adapted melphalan (MEL) and autologous stem cell transplantation (SCT) is an option for such patients.

We report on 12 patients, 8 men and 4 women with a median age of 58 (range, 53-70) with suboptimal responses to initial therapy who were referred for and underwent SCT after a median of 4 cycles of bortezomib-based therapy. Eight patients had NR with median involved FLC values at diagnosis and post initial therapy/pre-SCT of 180 (74-648) and 265mg/L (116-410) respectively while 4 had PR with medians of 1401 (250-9750) and 94mg/L (43-1620); in neither case were comparisons significant by paired t-test. Seven patients had 2 organs involved and 5 had one. Eight patients had cardiac involvement, seven stage 2 and one stage 3. Six patients had renal involvement, one stage 1, four stage 2, and one stage 3. Two had GI involvement. All patients were mobilized successfully with filgrastim and plerixafor as previously described. In SCT, four received MEL 200 mg/m2, seven MEL 140 mg/m2 and one MEL 100 mg/m2. All patients engrafted and all had their post-SCT hematologic responses assessed. There was one treatment-related death at 2 months post-SCT in a 70 year old woman who had stage 2 cardiac and stage 2 renal disease, received MEL 100 mg/m2 and had improved her hematologic response status from NR to PR. Overall post-SCT, 75% of patients achieved > VGPR including 42% who achieved CR (n=5). Eight patients have received consolidation with bortezomib and/or lenalidomide based regimens, with four patients achieving CR. Two patients are about to start consolidation. With a median follow-up of 27 months, two renal and four cardiac responses have been achieved. Median overall survival post-SCT has not been reached.

In conclusion, risk-adapted melphalan and SCT is a safe, feasible, timely and effective second-line therapy for patients with AL who have suboptimal responses to bortezomib-based initial therapy.

Randall-type MIDD: The disease spectrum

Florent Joly 1, Camille Cohen 2, Vincent Javaugue 1, Bertrand Arnulf 3, Bertrand Knebelmann 2, Bruno Rover 4, Mathilde Nouvier 5, Vincent Audard 6, Dominique Nochy 7, Francois Provot 8, Arnaud Jaccard 9, Guy Touchard 1, Celine Debiais Delpech 10, Jean Paul Ferrand 1, Frank Bridoux 1

1 CHU Poitiers, Nephrology, Poitiers, FRANCE 2 Hôpital Necker, Nephrology, Paris, FRANCE 3 Hôpital Saint Louis, Immunology and Hematology, Paris, FRANCE 4 CHU Amiens, Hematology, Amiens, FRANCE 5 CHU Lyon Sud, Nephrology, Lyon, FRANCE 6 Hôpital Henri Mondor, Nephrology, Creteil, FRANCE 7 HEGP, Pathology, Paris, FRANCE 8 CHU Lille, Nephrology, Lille, FRANCE 9 CHU Limoges, Hematology, Limoges, FRANCE 10 CHU Poitiers, Pathology, Poitiers, FRANCE.

Joly.floren@gmail.com

INTRODUCTION AND AIMS: Monoclonal immunoglobulin deposition disease (MIDD) is a rare complication of plasma cell disorders, defined by linear Congo red-negative deposits of monoclonal light chain (LCDD), heavy chain (HCDD) or both (LHCDD) along basement membranes. Renal involvement is prominent. To date, treatment strategies and predictors of global and renal survival in MIDD are poorly defined.

METHODS: We retrospectively reviewed 176 patients (male = 89) with biopsy-proven LCDD (n=143), HCDD (n=18) and LHCDD (n=15) in France between 1981 and 2014. Hematological response (HR) was assessed based on the difference between the involved and non involved light chain (dFLC). Renal response was defined as > 50 % decrease in 24h-proteinuria without a > 25 % decrease in eGFR. Patient and renal survival were estimated using the Kaplan-Meier method.

RESULTS: Median age was 63.3 years. All presented with renal involvement. Median Baseline eGFR was 18 ml/min/1.73m², with median proteinuria of 1.8 g/d, microhematuria in 68%, and hypertension in 64%. 55 patients had symptomatic extra-renal involvement, including heart (n=18), peripheral nerve (n=18) and liver (n=8) involvement. Hematologic diagnosis was symptomatic multiple myeloma (n=74, 42%), monoclonal gammapathy of renal significance (MGRS, n=100 (56.8%), waldenstrom macroglobulinemia (n=1), non-hodgkin lymphoma (n=2). Abnormal free light chain (FLC) ratio was found in all tested patients (113/113). Chemotherapy adapted to the underlying clone was given in 169 (96%) patients, including bortezomib in 93 (55%) patients, and alkylating agent-based therapy in 92 (55%). HR was obtained in 60% of patients. Median overall survival was 169 months in patients with post-treatment dFLC < 40 mg/L and 78 months in patients who did not achieve HR (p=0.001). Death-censored median renal survival was 216 months in patients with dFLC < 40 mg/L, and 108 months in those who did not reach HR (p=0.05). Renal response was associated with improved patient and renal outcomes. Indeed, median overall survival was 169 months in renal responders and 64 months in patients without a renal response (p=0.001). Death-censored median renal survival was 209 months in renal responders, compared to 109 months in non-responders (p=0.01). In univariate analysis, predictive factors of renal response were pre-treatment eGFR > 30 ml/min/1.73 m², post-treatment dFLC < 40 mg/L, bortezomib-based therapy, and absence of moderate to severe interstitial fibrosis and/or degenerative vascular lesions on kidney biopsy.

CONCLUSIONS: These data strongly support a role for chemotherapy introduced early during the course of Randall-type MIDD. Achievement of post-treatment dFLC <40 mg/L appears as the goal of chemotherapy, as it is associated with improved renal and patient survival. Renal response is a favorable prognostic factor of overall survival in MIDD. Novel anti-myeloma agents, particularly the proteasome inhibitor bortezomib, should be used as first-line because of their efficacy and good tolerance profile in patients with impaired kidney function.
Localized amyloidosis: a focal disorder without requirement for systemic therapy

Gaurav Sutrave¹, Peter Mollee², Simon Gibbs³, Simon Gibbs³, Ming-Wei Lin¹, Anthony Schwarer³, Graeme Stewart¹, Fiona Kwok¹

¹Westmead Amyloidosis Clinic, Westmead Hospital, Sydney, Australia. ²Princess Alexandra Hospital Amyloidosis Centre, Brisbane, Australia. ³Victorian and Tasmanian Amyloidosis Service, Monash University Eastern Health Clinical School, Melbourne, Australia. ⁴Australian Amyloidosis Network.

INTRODUCTION: Localized amyloidosis can be defined as amyloid fibril production and deposition confined to a single organ or tissue. It is a poorly understood entity. The aim of this study was to characterize the demographics, clinicopathologic features and prognosis of localized amyloidosis in an Australian cohort.

MATERIAL & METHODS: A case cohort analysis was performed of the patient database and medical records of all localized amyloidosis patients seen in the Australian amyloidosis clinical network. Patients had biopsy proven amyloidosis involving one organ where systemic AL, wtTTR, inherited and AA amyloidosis had been rigorously excluded.

RESULTS: 63 patients with localized amyloidosis were identified. Diagnosed between 1995 and 2015, the female: male ratio was 1.0:1.5 and median age 52 years. Median follow-up was 29 months. The most commonly involved sites were the upper respiratory (n=13) and genitourinary tracts (n=10). Clinical presentation was related to local mass effect or bleeding. No cases had heart, liver or kidney disease. The majority had light chain immunoglobulin as the constitutive amyloid protein when subtyped by mass spectrometry (n=16/17). The majority was presumed to arise from tissue-based clonal plasma cells. A minority (n=5) were localized to sites associated with a clonal lymphoproliferative disorder, most commonly MALT (n=4/5). Rare cases of non-clonal disorders were diagnosed using clinicopathologic means (n=3 semenogelin amyloid, n=1 insulin amyloid). Treatment was limited to focal surgical intervention using appropriate modalities. Despite persistent progressive disease in many, no patients have died and the majority did not require further intervention beyond the diagnostic biopsy (n=37/57 evaluable). None had progression to systemic amyloidosis, n=2 required systemic chemotherapy for progressive lymphoma, n=1 developed MGUS and n=1 Hodgkin’s disease.

DISCUSSION & CONCLUSIONS: Localized amyloidosis is a rare entity of single organ involvement consisting of a variety of constitutive amyloidogenic proteins but most commonly light chain immunoglobulin. Thorough testing is required to exclude systemic amyloidosis. Localized amyloidosis has significantly better prognosis than systemic AL amyloidosis and does not require systemic chemotherapy with local surgical intervention usually providing good disease control.
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**AL amyloidosis patients with a dFLC <50 mg/l at first diagnosis show unique characteristics, excellent hematologic response and favourable overall survival.**

T Dittrich, U Hegenbart, T Bochtler, C Kimmich, A Jauch, A Kristen, H Goldschmidt, AD Ho, SO Schönland

*Amyloidosis Center, University Hospital Heidelberg, Heidelberg, Germany*

Stefan.Schoenland@med.uni-heidelberg.de

**INTRODUCTION:** Systemic light chain amyloidosis (AL) is a rare and life-threatening protein-deposition disorder. The diagnosis and especially quantification of the underlying, usually small clonal B cell disorder in patients with very low levels of free kappa or lambda light chains in serum (FLC) can be challenging. DFLC (difference of involved minus uninvolved FLC) response to therapy is hardly assessable for initial values below 50 mg/l. We addressed this issue by characterization and systematic response assessment of AL amyloidosis patients presenting with initial dFLC <50.

**MATERIAL & METHODS:** We have retrospectively analysed clinical features, hematologic response and long-term outcome of 610 newly diagnosed AL patients with available dFLC and cytogenetic evaluation by iFISH at their first visit to our center between 2003-2015.

**RESULTS:** Patients with dFLC <50 showed lower bone marrow plasma cell counts (7 vs. 10, %, p <0.001), M-spike (6 vs. 9, g/l, p=0.006) and concentrations of the monoclonal heavy chain (7 vs. 10, g/l, p=0.011), while the mere presence of a monoclonal heavy chain in immunofixation (IF) was more frequent (51 vs. 39, %, p=0.038). All analysed chromosomal aberrations were not associated with dFLC <50. Patients with cardiac (42 vs. 82, %, p <0.001) and soft tissue (25 vs. 42, %, p=0.002) involvement, higher Mayo Score and lower Karnofsky Index were much less frequently found in the group with initial dFLC <50, while kidney involvement was more common (85 vs. 59, %, p <0.001) and more severe (higher renal stage, p=0.003). Notably, this was predominantly caused by higher initial proteinuria (6.6 vs. 1.7, g/d, p <0.001; but GFR: 73 vs. 63, ml/min) and did not translate into worse renal survival. Hematologic response after first-line therapy was deeper (CR after 3 and 6 months: 6 vs. 27 and 8 vs. 41, %, p <0.001) and median overall survival (OS) was significantly better in patients with dFLC <50. This was regardless of treatment type (Fig.).

**DISCUSSION & CONCLUSIONS:** AL patients with initial dFLC <50 mg/l represent a distinct clinical entity with predominance of renal involvement and less advanced heart disease. The underlying plasma cell clone is small and frequently presents a monoclonal intact heavy chain. Importantly, this is not associated with any particular chromosomal aberrations as revealed by iFISH. However, this group of patients show excellent hematologic response rates, which translates into very favourable OS irrespective of primary treatment regimens. Therefore, results of prospective clinical trials might be adversely influenced by the exclusion of these patients.

**Fig.:** Overall survival of 610 patients with dFLC <50 mg/l versus dFLC > 50 mg/l, grouped by treatment: Bortezomib-containing ("Bortezomib", medians: not reached vs. 16 months), Melphalan-containing (“Melphalan”, medians: 97 vs. 19 months) and high-dose Melphalan followed by autologous stem cell transplantation (“HDM + ASCT”, medians: not reached vs. 99 months).
Bortezomib-High Dose Melphalan conditioning is a feasible strategy for the treatment of transplant-eligible AL amyloidosis

VH Jimenez-Zepeda1, P Duggan1, P Neri1 and JB Bahlis1

1Department of Medical Oncology and Hematology, TBCC, Calgary, AB, Canada

Victor.Zepeda@albertahealthservices.ca

Introduction
Preclinical and clinical data suggest that bortezomib in combination with high-dose melphalan (Bor-HDM) provides with a synergistic effect able to improve the quality of response for MM and AL amyloid patients undergoing auto-SCT. In the present study, we aimed to assess the impact of Bor-HDM conditioning on ORR, and MRD negativity for AL patients undergoing single auto-SCT at our Institution.

Methods
All consecutive patients who underwent single auto-SCT at Tom Baker Cancer Center (TBCC) from 01/2010 to 12/2015 using Bor-HDM were evaluated. The diagnosis of AL amyloidosis and assessment of hematological and organ response was performed based on the consensus criteria published in 2005 and modified in 2012. MRD negativity was assessed by multiparameter flow cytometry. All patients received a median of 2 cycles of induction chemotherapy before undergoing auto-SCT (CyBorD). Bortezomib was administered intravenously at 1-1.3 mg/m² on days −5, −2, 1, and 4, while melphalan was given at 200 mg/m² on day −1.

Results
Four patients receiving Bor-HDM were evaluated. Clinical characteristics are listed in Table 1. At day-100 post auto-SCT, a ≥VGPR was seen in 75% (3 out of 4). A complete hematologic response was seen in 2 patients, this associated to MRD negative results in the bone marrow analysis. In addition, 1 patient exhibited VGPR with MRD negativity and the last patient only achieved PR but after 3 cycles of consolidation upgraded the response to VGPR; MRD + results were noted for this case at day-100 post-ASCT. All four patients exhibited an organ response at a median of 6 months post-ASCT. Furthermore, both patients who achieved CR also developed oligoclonal disease after ASCT. At the time of analysis, all patients are alive and progression-free with a median follow-up of 24 months. Time to engraftment and length of hospitalization was similar to that reported for our historical cases. With regards to toxicity, three-patient developed moderate to severe diarrhea and one patient tested positive for C.difficile and was treated successfully. In addition, one patient developed an episode of rash that required of prednisone treatment.

In conclusion, Bor-HDM is a safe and efficacious conditioning regimen able to increase the rate of complete hematologic response for patients with AL amyloidosis undergoing ASCT. Further studies are warranted to explore this regimen, especially when upfront therapies are employed, with special view on the toxicity and the role of MRD negativity acquisition. To the best of our knowledge this is one of the first reports on the use of MRD techniques in the assessment of response for AL amyloidosis. The impact of MRD negativity should be evaluated prospectively as novel and efficacious therapies continue to emerge.

REFERENCES


Table 1. Clinical and Laboratory characteristics of patients with AL Amyloidosis treated with bortezomib-HDM followed by ASCT at Tom Baker Cancer Center

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Bortezomib-HDM (n=4)</th>
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<tr>
<td>Age (years) median</td>
<td>60</td>
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<tr>
<td>Gender</td>
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<tr>
<td>Male</td>
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<tr>
<td>Female</td>
<td>50%</td>
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<tr>
<td>Mayo Clinic Staging</td>
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<td>Stage I</td>
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<td>&gt;3 organs involved by AL</td>
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<td>Alkaline phosphatase (Unit/L)</td>
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<td>Intraventricular Septal distance (mm)</td>
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<td>Ejection fraction (%)</td>
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<td>High-sensitive Troponin-T (ng/L) (normal 1-14)</td>
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<tr>
<td>NT-Pro-BNP (ng/L) (normal &lt;300)</td>
<td>271.5</td>
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</table>

BMPC: Bone marrow plasma cells: N/A (Not available)
Acquired von Willebrand Syndrome associated to secondary IgM MGUS emerging after Autologous Stem Cell Transplantation for AL Amyloidosis

VH Jimenez-Zepeda¹, H Qamar², L Skeith³ and K Valentine¹

¹Department of Medical Oncology and Hematology, TBCC, Calgary, AB, ²Department of Medicine, University of Alberta, Edmonton, and ³Department of Medical Oncology and Hematology, Ottawa, ON

INTRODUCTION: Acquired von Willebrand syndrome (AVWS) is a rare hemorrhagic disorder that often occurs in patients with no prior history of bleeding. It has been recognized that up to 23% of cases of AVWS is associated with monoclonal gammopathies. Here, we describe an interesting case of AVWS occurring 4-months post-autologous stem cell transplant (ASCT). MATERIAL & METHODS and RESULTS: A previously healthy, 56-years/old male presented with asymptomatic hypoalbuminemia detected on routine screening, later on patient went onto develop asymmetric inflammatory polyarthritis (MCP’s, wrists and shoulders) and proteinuria. Further investigations led to the diagnosis of AL Amyloidosis (Stage I) with a concurrent low grade B-cell neoplasm on bone marrow and no evidence of lymphadenopathy (22% of CD19+, CD20+, CD5- and CD10- with lambda restricted lymphocytes). Cardiac MRI and echocardiogram did not suggest amyloid involvement. Treatment with cyclophosphamide, bortezomib and dexamethasone (CyBorD) was initiated, achieving very good partial response (VGPR) after 3 cycles of therapy. Stem cell mobilization was successfully performed with G-CSF alone and subsequently autologous stem cell transplantation with bortezomib/melphalan conditioning was performed. At Day-100, response assessment was consistent with complete hematologic response. Four-months post-ASCT patient presented to ER with prolonged epistaxis. Prior to this time, he has had no history of bleeding. Laboratory investigations revealed prolonged PTT (45.5 s), fibrinogen 5.7 g/L (normal range 1.6-4.1), inhibitor screen negative, lupus type inhibitors negative, von Willebrand factor activity (GP1b) 0.12 U/mL (normal range: 0.41-1.44), von Willebrand factor Ag LIA 0.31 U/mL (normal range: 0.4-1.85), factor VIII activity 0.14 (normal range: 0.54-1.47), factor IX activity 1.02 U/mL (normal), factor XI activity 0.86 U/mL (normal), factor XII activity 0.3 (normal range: 0.48-1.6), factor X activity 0.91 U/mL (normal), factor XIII screen normal, Hb 75 g/L, Platelets 110 x10⁵, creatinine 139 umol/L, ANA-, RF-, Hep B and Hep C negative, cryoglobulin and agglutinin testing negative. Based on these investigations, the patient was diagnosed with Acquired von Willebrand syndrome (AVWS). No coagulation factor replacement was required and DDAVP challenge test demonstrated a good increase in von Willebrand factor and factor VIII levels, without rapid clearance. Patient also received prednisone at 1mg/Kg with slow dose tapering. Since that episode, no further bleeding has been reported and normalization of von Willebrand factor activity and antigen is maintained at 12 months. (Fig. 1) DISCUSSION & CONCLUSIONS: This case report illustrates the emerging complications for patients with AL amyloidosis receiving bortezomib-containing regimens prior to stem cell transplantation. Further data in this regard is needed to better understand the mechanisms by which these complications occur and how to minimize the morbidity related to these events.

REFERENCES

Figure 1. Levels of von Willebrand factor VIII Ag and Ristocetin Cofactor activity. (In the X axis, days from episode of bleeding is shown, while in the Y axis levels in U/mL are recorded)
Once weekly subcutaneous bortezomib, cyclophosphamide, and dexamethasone as induction therapy for all AL amyloidosis

H Abbas1, L Rybicki2, F Reu2, C Samaras2, M Smith2, D Hastings2, J Valent2

1Department of Internal Medicine, Cleveland Clinic, 2Department of Haematology and Oncology, Cleveland Clinic

Introduction: High dose melphalan followed by autologous stem cell transplant is the standard of care for all transplant eligible AL amyloidosis patients. Due to medical insurance approvals and pre-transplant evaluation, treatment is delayed by months. Given that there are many patients who respond quickly to bortezomib based regimens, we have adopted a bortezomib based induction regimen in an attempt to prevent early progression or death due to amyloidosis.

Materials and methods: All AL amyloidosis patients treated at the Cleveland Clinic who received bortezomib 1.3 mg/m2 subcutaneous, cyclophosphamide 300mg/m2 (dose cap at 500 mg) intravenous or oral, and dexamethasone 20 mg intravenous or oral (CyBorD) on day 1, 8, and 15, of a 28 day cycle were included in this analysis. Patient information was obtained from an institutional review board approved database and electronic medical record review. Hematologic response was assessed every 28 days. Transplant eligible patients who achieved early complete hematologic response (CR) completed up to 6 cycles of CyBorD followed by high dose melphalan and autologous stem cell transplant (ASCT). If there was no CR at the end of 2 cycles of therapy, patients underwent high dose melphalan and ASCT. Non- transplant eligible patients completed a planned 6 cycles of CyBorD. The primary outcome of the study was to assess overall survival (OS) of all AL amyloidosis patients by Kaplan-Meier estimate. The secondary outcomes were to assess the best hematologic response1, with CyBorD plus ASCT versus CyBorD alone and evaluate if any transplant eligible patients were unable to proceed to ASCT after receiving CyBorD.

Results: With a median follow up of 30 months, patients who received CyBorD plus ASCT had a 92% probability of survival. Patients who received CyBorD alone had a 47 % probability of survival. All patients who were deemed transplant eligible at diagnosis underwent high dose melphalan and ASCT. The median NT-proBNP for patients who underwent ASCT was 305 pg/ml and for patients who were transplant ineligible was 6047 pg/ml. The best hematologic response in patients who received bortezomib based induction plus transplant was CR 71%, very good partial response (VGPR) 18%, stable disease (SD) 6%, and progressive disease (PD) 6%. Patients treated with CyBorD alone had a CR 56%, VGPR 6%, partial response 6%, SD 19%, and PD 12%.

Discussion: None of the transplant eligible patients were deemed transplant ineligible after the induction therapy. The OS was excellent for patients who underwent transplant. The percentage of CR in the transplant eligible patients compared favourably to historical reports of CR with ASCT alone. Only 12 % of patients undergoing ASCT failed to achieve a VGPR compared to 37% of patients with CyBorD alone. Certainly the transplant ineligible patients had severely compromised baseline cardiac function but despite that the probability of survival at 30 months was 47%. The use of CyBorD during preparation for ASCT does not compromise transplant eligibility and may increase hematologic response rates in AL amyloidosis patients.

Abbreviations: NT-proBNP (amino-terminal pro-B-type natriuretic peptide).

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Lessons for future clinical trial design in cardiac amyloidosis - the experience of a prospective study in stage III cardiac AL amyloidosis (REVEAL study)

AD Wechalekar1, P Smith2, CJ Whelan1, M Fontana1, S Mahmood1, HJ Lachmann1, T Adedayo2, JD Gillmore1, PN Hawkins1 and S Nash2

National Amyloidosis Centre, Dept of Medicine, University College London, London1
Cancer Research UK and University College London Cancer Trials Centre, London2
a.wechalekar@ucl.ac.uk

Prospective clinical trials in AL amyloidosis are difficult due to the rarity of the disease and challenging clinical course of the condition. The arrival of bortezomib in the mid-2000’s marked the beginning of an era of novel agents for treatment of AL amyloidosis. CAN-001 was a prospective trial of single agent bortezomib with addition of dexamethasone, in patients with relapsed refractory disease. We and colleagues reported our retrospective experience with bortezomib in the same setting (Kastritis et al JCO 2008). REVEAL was conceived as a trial for bortezomib triplet combination in AL amyloidosis. The course of this clinical trial elucidates the challenges in conducting a trial for a condition where high mortality is part of the disease course.

This trial design was conceived in 2008 and was submitted for peer review as a single arm trial of bortezomib triplet in relapsed disease. Based on Vincristine-Adriamycin-Dexamethasone (VAD) as the standard of care for front line treatment of AL amyloidosis in all but the very advanced cases in UK and the early reports of high efficacy of Bortezomib with Adriamycin/Dexamethasone (PAD), PAD was chosen as the study arm. The peer review and funding application went through various iterations over 2 years by which time bortezomib became routinely available for relapsed myeloma in the UK and use of bortezomib triplets became common – both were adopted for relapsed AL. Hence the setting was changed to upfront treatment in a parallel phase II trial of PAD and Cyclophosphamide-Bortezomib-Dexamethasone (CVD). Each arm was independent to allow for separate stopping rules. The funding for the trial was finally approved in 2011 and the trial opened to recruitment in 2012 for stage III cardiac AL.

The trial opened in March 2012 and four patients were recruited by May 2012 (one patient on the CVD arm and three on the PAD arm). Three patients died – one on the CVD arm and two on the PAD arm. The last patient on the PAD arm was discontinued due dexamethasone induced psychosis. The trial was halted for a safety review. The data was presented to the independent data monitoring committee (IDMC) which concluded the deaths to be multifactorial - mainly disease related, but the impact of the drugs could not be ruled out. Suggested modifications went back and forth between the sponsor, the IDMC (and later the manufacturer of bortezomib) – the trial eventually reopened in April 2013 (after 11 months) modified as VD vs. CVD. The IDMC wanted safety reporting after every three patients. A further 3 patients were recruited – there was one death in the CVD arm. Although this was within the “expected” outcome for patients with cardiac AL amyloidosis, the IDMC again stopped the trial in Oct 2013. The trial design was changed as VD vs. a single dose escalating bortezomib arm as per discussions with the IDMC. This design was considered as a substantial change in trial design by the regulatory authority needing full regulatory re-submission. Due to the fact that over 200 patients had received upfront bortezomib (mainly CVD) outside the trial, the trial management group decided to close the trial in late 2014.

With availability of novel chemotherapy agents and drugs that accelerate amyloid fibril removal, studies in upfront cardiac AL amyloidosis are being designed. Building safeguards at each level, educating the independent data monitors, regulators and funders to the complexity of AL and “expectedness” of events is critical to avoid the pitfalls that lead to prolonged delays at every stage of this trial - leading to eventual closure of the study.
PB108

SPECKLE TRACKING STRAIN FOR EARLY DETECTION OF RIGHT VENTRICULAR SYSTOLIC AND DIASTOLIC DYSFUNCTION IN CARDIAC AMYLOIDOSIS

EM Chia, Q Lo, F Kwok, MW Lin, G Stewart, D Leung, DAB Richards, L Thomas

1 Department of Cardiology, Liverpool Hospital, Sydney, Australia. 2 Westmead Amyloidosis Clinic, Westmead Hospital, Sydney, Australia

eemayc@gmail.com

INTRODUCTION: Left ventricular (LV) cardiac involvement in light chain (AL) amyloidosis is associated with poor prognosis. More recently, right ventricular (RV) involvement has also shown prognostic value, albeit in a small patient group. Speckle tracking strain is useful in RV assessment as it is angle independent. We sought to evaluate global and segmental RV systolic and diastolic function in AL amyloidosis patients with cardiac (CA) and without known cardiac (NCA) involvement and to determine the influence of LV diastolic dysfunction (DD) on RV function. We additionally evaluated its relationship to biomarkers such as NT-proBNP.

METHODS: We prospectively recruited 57 biopsy proven AL amyloidosis patients from a tertiary referral centre. Patients were followed up for a mean of 60 months. Detailed transthoracic echocardiograms were performed with particular emphasis on the right ventricle. RV systolic (RV fractional area change (FAC), tricuspid annular plane excursion (TAPSE) and s’ velocity) as well as diastolic function (RV e’ and a’ velocities, E/e’ and E/A ratio) were obtained. RV strain and strain rate were obtained from a RV focused view. Patients were stratified as having CA if the mean value of LV wall thickness was ≥ 12 mm, or NCA if <12 mm as previously described. They were compared with age and sex matched healthy controls.

RESULTS: 22/57 (39%) of patients were classified as NCA. Despite normal LV and RV wall thickness, there was decreased RV systolic function and diastolic function as evidenced by a decrease in RV strain, E and A strain rate. RV strain was a predictor of mortality (χ²=4.07, p=0.04), however, RV strain in combination with a raised NT-proBNP was an even better predictor (χ²=8.84, p=0.003). With increasing grade of LV DD, there was a decline in RV systolic and diastolic function.

DISCUSSION & CONCLUSIONS: Subclinical RV systolic and diastolic dysfunction by strain analysis is seen in NCA patients prior to the development of LV hypertrophy or significant LV DD using RV strain analysis. AL amyloidosis affects both the left and right ventricle; careful biventricular assessment is therefore warranted in all patients for early diagnosis of cardiac involvement.


Table 1: Amyloid subgroups versus Controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>NCA</th>
<th>CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT-proBNP (ng/L)</td>
<td>N/A</td>
<td>173±341</td>
<td>1299±2809</td>
</tr>
<tr>
<td>RV TAPSE (mm)</td>
<td>20.9±1.9</td>
<td>20.2±6.3</td>
<td>17.2±4.2*</td>
</tr>
<tr>
<td>RV s’ (cm/s)</td>
<td>11.3±1.6</td>
<td>10.4±3.5</td>
<td>9.5±2.3*</td>
</tr>
<tr>
<td>Strain R Ave (%)</td>
<td>-25.9±4.9</td>
<td>-20.8±6.0*</td>
<td>-17.6±7.3*</td>
</tr>
<tr>
<td>E-Sr (s-1)</td>
<td>1.67±0.50</td>
<td>1.31±0.52*</td>
<td>1.23±0.49*</td>
</tr>
<tr>
<td>A-Sr (s-1)</td>
<td>1.52±0.41</td>
<td>1.13±0.61*</td>
<td>1.22±0.75*</td>
</tr>
</tbody>
</table>

Table Caption: *p<0.05 when compared with controls, § p<0.05 when compared with NCA patients
PB109

Immunochemical detection of light chain in tissue amyloid deposits combined with serum free light chain dimerization pattern as a diagnostic tool in ambiguous cases with plasma cell dyscrasia – report of 10 cases manifested with kidney disease

O. Kukuy 1, A. Livneh 2,3, A. Duek 4, M. Leiba 3,4, E. Ribakovski 4, A. Volkov 5, G. Schiby 5, B. Kaplan 2

1 Institute of Nephrology and Hypertension, Sheba Medical Center, Tel Hashomer; 2 Laboratory of FMF and Amyloidosis, Heller Institute of Medical Research, Sheba Medical Center, Tel Hashomer; 3 Internal Medicine Division, Sackler School of Medicine, Tel-Aviv University, Tel Aviv; 4 Division of Haematology and Bone marrow Transplantation, Sheba Medical Centre, Tel-Hashomer; 5 Institute of Pathology, The Chaim Sheba Medical Center, Tel-Hashomer

lesya.kukuy@gmail.com

INTRODUCTION: Plasma cell dyscrasia (PCD) may present with diverse kidney pathology. Thus, the finding of proteinuria ± renal impairment ± kidney histology may not be enough to determine the type and status (remission or relapse) of the PCD. Previously we reported an immunochemical method for detection of light chain deposits in tissues, while recently we showed that analysis of serum free light chain (FLC) monomers (M) and dimers (D) might be helpful in differentiating between malignant and benign forms of PCD. The aim of the study was to evaluate the performance of our method for detection of light chain in tissue amyloid deposits, combined with characterization of serum FLC dimerization pattern in the diagnosis and management of obscure cases of PCD.

MATERIAL & METHODS: In this retrospective study, we enrolled all patients with renal disease and PCD referred to us over the last 5 years (2010 to 2015) for amyloid typing or for PCD diagnosis. All patients failed routine laboratory testing, commonly employed to address these diagnostic dilemmas. Extraction of amyloid proteins from tissues and their typing was performed as published previously (Kaplan et al, 1999, 2004, 2005, 2009). FLC M-D analysis was carried out by semi-quantitative Western blotting, based on SDS-electrophoresis under non-reducing conditions.

RESULTS: Of the 10 patients (5 men) with kidney disease manifested with proteinuria ± renal impairment ± PCD referred to us, 4 had monoclonal kappa and 3 monoclonal lambda FLC (by routine FLC nephelometry). In 3 amyloidosis patients no monoclonal light chain was detected by nephelometry or immunofixation. The kidney pathology findings comprised of amyloidosis (5 cases) and glomerulonephritis (2 cases). Two patients with proteinuria had amyloidosis diagnosed by gastrointestinal tract biopsy and one by skin biopsy. The final diagnoses, attained by our methodology and confirmed by patients follow up, was AL amyloidosis (6 cases), monoclonal gammopathy of undetermined significance (MGUS) unrelated to kidney pathology (2 cases), multiple myeloma (MM) unrelated to kidney pathology (1 case) and a patient with AL amyloidosis in remission that had normal FLC dimerization pattern (1 case).

DISCUSSION & CONCLUSIONS: combined use of our tests for light chain tissue deposits and for serum FLC dimerization patterns appears as an important ancillary aid in diagnosis of challenging cases with PCD.

Patient-reported outcomes in light chain amyloidosis: choosing the optimal tool

Anita D’Souza1, Marcelo Pasquini1, Parameswaran Hari1, Kirsten Jacobson1, Kathryn Flynn2

1Division of Hematology/Oncology, 2Patient Care and Outcomes Research

Department of Medicine
Medical College of Wisconsin, Milwaukee, WI

Introduction: Light chain amyloidosis (AL) is a rare blood disease with multisystem involvement. Treatment of AL is targeted at the underlying neoplasm using chemotherapies (such as bortezomib, lenalidomide, alkylators, steroids) or autologous stem cell transplant in select patients. Chemotherapy, however, can only target the underlying neoplasm but has no effect on already damaged organs. Symptoms of AL depend on the affected organs, making the amyloid symptom experience highly varied across patients. Moreover decrements in symptoms and function caused by the disease can overlap with those caused by treatment. There is very limited literature on the patient’s experience in AL, and no established approach to measuring patient-reported outcomes (PROs)(1). We recently began a research program to better understand the AL patient experience, including choosing the optimal PRO tool.

Methods: We included the Patient Reported Outcomes Measurement Information System (PROMIS) Global Health item, a 10 question tool, in a prospective phase 2 clinical trial assessing the utility of doxycycline as an amyloid de-fibrillogenic agent which may be combined with chemotherapy in patients with AL amyloidosis.

Results: The 14 patients enrolled on trial thus far include 3 patients with localized AL and 11 with systemic AL. Of the domains covered in the PROMIS Global Health measure, physical function and fatigue appeared to be the most commonly affected in AL patients at the time of diagnosis. There was evidence of variability in patients’ experiences across all domains. The average PROMIS Global Physical Health T score was 41.9 and the average Global Mental Health T score was 45.7 (compared to mean score of 50 for the general population, and <50 signifying worse PRO).

Discussion: We expect widely variable domains to be affected and differentially change over time among AL patients since the AL experience is dependent on the organs affected and the severity of organ involvement. Thus we determined we would be better served by a measure that scores domains individually rather than an index. Even with a 10 question survey, we are able to show declines in the physical health and mental health domains in patients. Longitudinal assessments which will be shown in the final presentation will allow assessing changes in scores during therapy.

Conclusions: In order to obtain more precision, we are considering either a customized PROMIS instrument comprising of either longer short forms or computerized adaptive testing (CAT) with the addition of items from the FACIT system to capture additional symptoms including dyspnea, edema, neuropathy, loss of appetite and diarrhea.

References:
Urinary albumin to creatinine ratio in diagnosis and risk stratification of renal AL amyloidosis

G Palladini, P Milani, M Basset, F Russo, A Foli, G Merlini

Amyloidosis Research and Treatment Center, Foundation Istituto di Ricovery e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, and Department of Molecular Medicine, University of Pavia, Pavia, Italy

giovanni.palladini@unipv.it

INTRODUCTION: In AL amyloidosis, the definition of renal involvement, the evaluation of severity of renal damage, and the assessment of renal response to therapy is based on measurement of 24 hour proteinuria (24hUP). However, collecting a 24 hour urine sample is cumbersome and prone to errors. The urinary albumin to creatinine ratio (UACR) has been proposed as a practical alternative to measure urine protein loss and is widely used by nephrologists. We evaluated the performance of UACR in diagnosing renal involvement and predicting renal outcome in patients with AL amyloidosis.

MATERIALS & METHODS: We prospectively measured 24hUP and UACR in 224 newly diagnosed (2013-2015) patients with AL amyloidosis. Patients were instructed to perform a 24h urine collection. The last morning void was used for UACR measurement. Correlation between UACR and 24hUP was assessed by Pearson’s r. ROC analyses were used to identify UACR cutoffs. Patients who died were censored for calculating renal survival defined as time to dialysis.

RESULTS: Sixty-four percent of patients had kidney involvement as defined by a 24hUP >0.5 g/24h (predominantly albumin). Renal stage based on 24hUP (cutoff 5 g/24h) and estimated glomerular filtration rate (eGFR, cutoff 50 mL/min per 1.73 m²) was I in 48% of cases, II in 37%, and III in 15%. Median (interquartile range) 24hUP and UACR were 1.7 g/24h (0.3-6.3 g/24h) and 1312 mg/g (98-6188 mg/g), respectively. There was a good correlation between 24hUP and AUCR (Pearson’s r = 0.90, 95%CI: 0.87-0.92). The best UACR cutoff for the diagnosis of renal involvement (defined as 24hUP >0.5 g/24h) was 500 mg/g (area under the ROC curve 0.94, 95%CI: 0.90-0.97; sensitivity 89%, 95%CI: 83-94%; specificity 97%, 95%CI: 91-100%). The definition of renal involvement with 24hUP and UACR was concordant in 92% of cases (95%CI: 88-95%). After a median follow-up of living patients of 8 months, 16 patients (7%) required dialysis. The UACR cutoff best discriminating patients who required dialysis at 6 months was 3600 mg/g. This was used to substitute the 24hUP cutoff (5 g/24h) in the renal staging system. There was a 90% concordance (95%CI: 86%-94%) in renal staging with the 24hUP and the UACR based staging systems. Both staging systems discriminated 3 groups with increasing risk of progression to dialysis (Figure 1A and B). A total of 103 patients had follow-up data at 6 months. A >30% reduction in UACR in the absence of >25% decreases in eGFR best predicted renal survival and was used to define renal response. Although the difference did not reach statistical significance, all progressions to dialysis occurred in non-responders defined either per standard criteria or using the novel UACR criteria.

DISCUSSION & CONCLUSIONS: These data indicate that UACR can be used to identify renal involvement, predict renal outcome and possibly assess renal response in AL amyloidosis. A longer follow-up in this series and validation in independent populations are warranted.
Severity and reversibility of cardiac dysfunction and residual concentration of amyloidogenic light chain predict overall survival of patients with AL amyloidosis who attain complete response

G Palladini, P Milani, M Basset, F Russo, M. Nuvolone, A Foli, G Merlini

INTRODUCTION: Complete response (CR) is considered the ideal target in the treatment of AL amyloidosis, being associated with best survival. However, some patients who attain CR have persistent relevant organ dysfunction that can be fatal, and some eventually relapse and die if treatment fails to restore response. In the present study we investigated the factors predicting the outcome of patients with AL amyloidosis who attained CR after upfront chemotherapy.

MATERIAL & METHODS: The prospectively maintained database of the Pavia Amyloidosis Research and Treatment Center, including 1069 treatment-naïve patients with AL amyloidosis diagnosed between 2004 and 2015 was searched for patients who reached CR defined as negative high-resolution immunofixation of serum and urine (IFE) and normal free light chain (FLC) ratio confirmed at 2 consecutive assessments.

RESULTS: A total of 122 patients were included. The kidney was involved in 79% of patients, the heart in 70%, the soft tissues in 19%, the liver in 14%, and the peripheral and autonomic nervous system in 13% and 9%, respectively. Cardiac stage was I in 30% of cases, II in 43%, IIIa in 20%, and IIIb in 7%. Renal stage was I in 46%, II in 38%, and III in 16% of patients. Median bone marrow plasma cell (BMPC) infiltrate was 10% (range: 2-45%). Most common treatments were melphalan/dexamethasone (MDex) in 41% of subjects, cyclophosphamide/bortezomib/dexamethasone in 29%, bortezomib/MDex in 12%. With the exception of transplanted patients (2%), all subject received 2 additional cycles after achievement of CR. With a median follow-up of living patients of 4.1 years, 30 patients (25%) died. Cumulative proportion survival was 87%, 81%, and 54% at 3, 5, and 10 years, respectively. At univariate analysis, the variables significantly predicting survival were baseline difference between involved (amyloidogenic, iFLC) and uninvolved FLC (best cutoff 90 mg/L, 5-year survival 93% vs. 72%, P=0.024), post-treatment iFLC (best cutoff 17 mg/L for λ clones [80%], 5-year survival 93% vs. 71%, P=0.012, not calculated due to small numbers in κ clones), baseline NT-proBNP >2700 ng/L, 5-year survival 95% vs. 64%, P<0.001), baseline cTnI (best cutoff 0.024 ng/mL, 5-year survival 96% vs. 74%, P=0.006), and achievement of a cardiac response (by NT-proBNP) at the time of CR (5-year survival 87% vs. 61%, P=0.047). Interestingly, baseline BMPC infiltrate and type of treatment did not predict survival. We designed competitive multivariate models including non-collinear clonal and cardiac variables. The best models are reported in Table 1. Severity and reversibility of cardiac dysfunction and residual iFLC were independent prognostic determinants.

DISCUSSION & CONCLUSIONS: These data emphasize the need of amyloid-targeting therapies increasing the rate of cardiac response even in patients in CR. The prognostic impact of low concentrations of iFLC suggests the persistence of clonal FLC in patients who fulfill current criteria for CR. New highly sensitive and specific tools for the detection of minimal residual disease in AL amyloidosis are necessary.

Table 1. Multivariate analysis of survival in patients with AL amyloidosis attaining CR after upfront therapy

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR (95% CI)</th>
<th>P</th>
<th>HR (95% CI)*</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>post-treatment iFLC (mg/L)</td>
<td>1.02 (1.01-1.04)</td>
<td>&lt;0.001</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>post-treatment iFLC &gt;17 mg/L</td>
<td>-</td>
<td>-</td>
<td>4.12 (1.00-17.58)</td>
<td>0.048</td>
</tr>
<tr>
<td>baseline NT-proBNP &gt;2700 ng/L</td>
<td>3.97 (1.33-11.85)</td>
<td>0.014</td>
<td>4.62 (1.35-15.84)</td>
<td>0.015</td>
</tr>
<tr>
<td>NT-proBNP response</td>
<td>0.28 (0.11-0.69)</td>
<td>0.006</td>
<td>0.42 (0.18-1.00)</td>
<td>0.050</td>
</tr>
</tbody>
</table>

*λ clones only (N=97)
Patterns of relapse after upfront bortezomib therapy in AL amyloidosis

M Basset, P Milani, F Russo, M. Nuvolone, M. A Foli, G Palladini, G Merlini

*Amyloidosis Research and Treatment Center, Foundation Istituto di Ricerca e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, and Department of Molecular Medicine, University of Pavia, Pavia, Italy*

giovanni.palladini@unipv.it

**INTRODUCTION:** In the last few years a major international effort allowed establishing baseline staging systems and response criteria in AL amyloidosis. However, we still lack validated progression criteria. This is acutely relevant for reporting progression free survival in clinical trials and because novel agents are first tested in the relapsed/refractory setting. We describe the characteristics and outcome of 130 consecutive patients with AL amyloidosis who responded to upfront bortezomib-based therapy.

**MATERIAL & METHODS:** The prospectively maintained database of the Pavia Amyloidosis Research and Treatment Center, including 1069 treatment-naïve patients with AL amyloidosis diagnosed between 2004 and 2015, was searched for patients who responded to upfront bortezomib-based therapy, and did not require second-line therapy for at least 6 months.

**RESULTS:** A total of 130 consecutive patients were identified. Their median age was 63 years. The kidney was involved in 74% and the heart in 68% of subjects. At baseline, renal stage was I in 41% of patients, II in 38%, and III in 21%. Cardiac stage was I in 26%, II in 46% IIIa in 20%, and IIIb in 8%. Upfront treatment was CyBorD in 55% of cases, BMDex in 35%, BDex in 8%, and BDR in 2% of subjects with IgM-AL amyloidosis. Best response at the time of bortezomib discontinuation was CR in 28%, VGPR in 59%, and PR in 13%. Cardiac response was achieved in 38% of patients and renal response in 27%. All patients in whom treatment was discontinued in PR had also achieved organ response. After a median follow-up of living patients of 33 months, 32 patients (25%) needed second line therapy (relapsed). Factors predicting time to relapse were baseline involved/uninvolved FLC ratio (IUR) >6 (HR 4.15, P=0.004), dFLC >60 mg/L (HR 4.74, P=0.011), and use of upfront melphalan (protective, HR 0.36, P=0.015). Achievement of CR and/or VGPR did not significantly predict time to relapse in this series, probably due to the small number of patients in PR. Since IUR and dFLC were collinear, we designed 2 alternative multivariate models including either IUR or dFLC and treatment with BMDex (Table 1). The reasons for starting second line therapy were increase in dFLC [reaching a median (range) of 60 mg/L (28-117 mg/L), corresponding to 36% (11-74%) of baseline value] in 30 cases (94%), NT-proBNP progression in 11 (34%, including 3 subjects who did not present with heart involvement at diagnosis), >50% increase in proteinuria in 7 (22%), and eGFR progression in 5 (16%). Overall, 34% of relapsing patients had no signs of organ progression. Seven patients died and median survival after relapse was 22 months. The only factor predicting survival after relapse was a NT-proBNP concentration >1650 ng/L (P=0.043).

**DISCUSSION & CONCLUSIONS:** In our practice, second-line therapy is almost invariably triggered by an increase in dFLC, which is accompanied by organ progression in three fourths of patients. Duration of response after bortezomib-based therapy is reduced by baseline clonal characteristics (high dFLC and IUR) and is prolonged by exposure to melphalan. Survival after relapse depends on persistent cardiac dysfunction, emphasizing the need of improving cardiac damage in patients who attain hematologic response.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR (95% CI)</th>
<th>P</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>involved/uninvolved FLC ratio &gt;6</td>
<td>3.09 (1.06-9.00)</td>
<td>0.039</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>dFLC &gt;60 mg/L</td>
<td>-</td>
<td>-</td>
<td>4.45 (1.06-18.74)</td>
<td>0.043</td>
</tr>
<tr>
<td>Treatment with BMDex</td>
<td>0.42 (0.18-0.95)</td>
<td>0.037</td>
<td>0.40 (0.18-0.90)</td>
<td>0.028</td>
</tr>
</tbody>
</table>
INTRODUCTION: The proportion of bone marrow plasma cell (BMPC) has been considered to have prognostic impact in patients with AL amyloidosis (1). The aim of this study was to analyse the correlation between the percentage of BMPC, clinical features and outcome in a series of patients from a single institution.

MATERIAL & METHODS: A series of 82 patients (42F/40M; median age 60 years-old) diagnosed with systemic AL amyloidosis at our institution between April 2008 and October 2015 was analysed. Clinical and laboratory data, treatment received, including autologous stem-cell transplantation (ASCT), and follow up data were available in all patients. Overall survival (OS) was estimated by the Kaplan-Meier method.

RESULTS: Median BMPC infiltration was 11% (range 1-90) with a significantly correlation to a higher serum free light chain (FLC) difference (p=0.015). 18 patients (22%) presented with 20% or higher BMPC infiltration. In the whole series, median OS was 5.1 years. None of our patients developed CRAB criteria or any other myeloma feature during the disease evolution. Early mortality (within one year from diagnosis) was higher for patients with BMPC >10% (27.3% vs. 11.4%, p=0.08). Cardiac involvement was also more prevalent in patients with higher BMPC infiltration (86.4% vs. 63%; p=0.015) whereas a lower BMPC infiltration was associated with non-life threatening organ involvement, mainly kidney (85.7% in patients with >10% vs. 44% in patients with >10% BMPCs; p <0.001). In patients with >10% BMPC involvement, the OS was significantly shorter (median 2.8 years vs. not reached, p=0.033) (Figure) and even lower BMPC thresholds had impact on OS (i.e. >7% BMPC, p=0.009). In a multivariate analysis, the prognostic impact of bone marrow involvement was not statistically independent from cardiac involvement.

DISCUSSION & CONCLUSIONS: In patients with AL amyloidosis, a higher bone marrow plasma cell involvement is associated with increased systemic organ damage, particularly cardiac involvement. This is not related to the presence or development of myeloma features.


Figure. Overall survival of patients with AL amyloidosis according to bone marrow plasma cell infiltration (BMPC)
Prevalence and prognostic impact of oligoclonal bands in patients with AL amyloidosis

C Fernández de Larrea1, LG Rodríguez-Lobato1, MT Cibeira1, N Tovar1, JJ Aróstegui2, LR Rosiñol1, M Elena3, JYagüe2 and JBładé1

Amyloidosis and Myeloma Unit. Department of 1Hematology, 2Immunology and 3Biochemistry. Hospital Clinic, Barcelona. Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). University of Barcelona. Barcelona, Spain.
cfernan1@clinic.ub.es

INTRODUCTION: The emergence of oligoclonal bands (OB) in patients with multiple myeloma (MM) achieving a complete response (CR) is a well-recognized event after autologous stem-cell transplantation (ASCT) and the use of novel agents (1). However, the frequency of clinical impact and outcomes of the emergence OB in patients with AL amyloidosis have never been described. The objective of this study is to determine the prevalence, natural history and prognostic impact of OB in patients with AL amyloidosis.

MATERIAL & METHODS: We reviewed the clinical records of patients with AL amyloidosis from January 1996 to September 2015. Fifty-five patients (26F/29M; median age at diagnosis 61 years) with PR or better after different induction regimens and/or ASCT were found. Initial baseline demographics, clinical and laboratory data, treatment and follow-up were collected. Median follow-up was 3.5 years. An OB was defined by the presence of a serum and/or urine immunofixation monoclonal spike different either in heavy and/or light chain component from the original monoclonal protein.

RESULTS: Thirty patients were transplant ineligible while 45.5% (25) had received an ASCT. We observed OB in 59% of the patients; median number of bands per patient was 2 (range 1 to 5). The most frequent isotypes were IgG-kappa (30%) and IgG-lambda (22%). The oligoclonal phenomenon was more prevalent in patients in CR compared to the other degrees of response (82.1% vs. 33.3%; p=0.0001). There were no statistical differences between the emergence of OB and type of treatment received (ASCT vs. chemotherapy alone; p=0.118) or involved isotype light chains (p=0.31). Regarding its prognostic value, the presence of oligoclonality lasting for more than one year resulted in a significantly longer overall survival (OS) as compared to patients without OB or with only a transient phenomena (p=0.013) (Figure).

DISCUSSION & CONCLUSIONS: This is the first report describing the prevalence of OB in patients with AL amyloidosis after first-line therapy, which is even higher than that observed in patients with MM. Patients with oligoclonal humoral response lasting for more than one year had a significantly longer OS, likely reflecting a more robust humoral immune response.

Amyloid load in fat tissue reflects severity of cardiac involvement and activity of plasma cell dyscrasias and is consecutively prognostic for survival in systemic light chain amyloidosis

C Kimmich¹, S Schönland¹, S Kräker¹, B Geisler¹, AD Ho¹, J Bijzet², B Hazenberg², U Hegenbart¹

¹Amyloidosis Centre, Department of Medicine V, University Hospital Heidelberg, Heidelberg, Germany. ²University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.
Christoph.Kimmich@med.uni-heidelberg.de

INTRODUCTION: Subcutaneous fat aspiration is a simple minimal-invasive technique to detect amyloid with high sensitivity and specificity (1). In addition, an association between amyloid load in fat tissue and its impact on survival was reported (2). We introduced this technique at the University Hospital Heidelberg in May 2013 with the support from the Amyloidosis Centre Groningen.

MATERIALS & METHODS: We prospectively analysed 183 untreated patients with biopsy-proven systemic light-chain (AL) amyloidosis between September 2013 and June 2015. Fat aspirates were analysed using a 100 watt polarisation microscope (Zeiss Axioplan 2) and graded according to involved surface area as presented (1): negative equals 0, below 1% equals 1+, 1 to 10% equals 2+, 10 to 60% equals 3+ and above 60% equals 4+. For simplification, we performed a split into abundant for 3+ and 4+ and non-abundant for 0, 1+ and 2+. Patients were also assessed clinically, received a broad laboratory analysis, an echocardiography and bone marrow (BM) diagnostics. Furthermore, for the majority of patients regular follow-ups were available (n=175). For organ involvement, we utilised the current consensus criteria (Tours), for cardiac staging, we classified according to (3).

RESULTS: Amyloid was detected in 94% (172/183) and was graded as 0+ in 11 (6%), 1+ in 38 (21%), 2+ in 40 (22%), 3+ in 36 (20%) and 4+ in 58 patients (32%). Patients graded as abundant had cardiac involvement significantly more often than patients graded as non-abundant (96% vs. 69%, p<0.001). Cardiac biomarkers and the markers for the underlying plasma cell dyscrasia were significantly higher for patients with abundant deposits compared to patients without these findings (for median values see Table 1). Patients classified as non-abundant had renal involvement significantly more often (63% vs. 40%, p<0.01). No correlation between severity of renal involvement and amyloid load could be detected. Females were classified as abundant significantly more frequently than men (n=47/78, 60% vs. n=47/105, 45%, p<0.05). Six months after fat aspiration 31% (28/91) of patients graded as abundant had died compared to only 12% (10/84) of patients graded as non-abundant (p<0.01).

DISCUSSION & CONCLUSIONS: Patients with abundant amyloid deposits in fat aspiration samples nearly always have cardiac involvement with severely increased cardiac biomarkers. Our findings suggest that abundant AL amyloid deposits in fat tissue reflect cardiac injury and the increase in mass. Survival at six-months is significantly reduced in patients with abundant amyloid deposits. To the contrary, we show that in renal AL amyloidosis no mass effect can be detected in subcutaneous fat tissue.


<table>
<thead>
<tr>
<th></th>
<th>Abundant</th>
<th>Non-Abundant</th>
<th>p-Value</th>
</tr>
</thead>
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<tr>
<td>NT-ProBNP in ng/l</td>
<td>6297</td>
<td>607</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>hsTnT in ng/l</td>
<td>55</td>
<td>23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Septal wall thickness in mm</td>
<td>16</td>
<td>13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dFLC in mg/l</td>
<td>305</td>
<td>96</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BM plasma cells in %</td>
<td>12</td>
<td>8</td>
<td>&lt;0.01</td>
</tr>
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<td>Albuminuria in mg/day</td>
<td>183</td>
<td>1768</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>eGFR</td>
<td>69</td>
<td>63</td>
<td>0.97</td>
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<tr>
<td>Age</td>
<td>64</td>
<td>66</td>
<td>0.64</td>
</tr>
</tbody>
</table>
Congored staining to detect amyloid in bone marrow aspirates – sensitivity in comparison to histology and fat aspiration - analysis of organ involvement patterns

C Kimmich¹, S Schönland¹, S Kräker¹, AD Ho¹, G Mayer¹, M Hundemer¹, U Hegenbart¹

¹ Amyloidosis Center, Department of Medicine V, University Hospital Heidelberg, Heidelberg, Germany.

Christoph.Kimmich@med.uni-heidelberg.de

INTRODUCTION: Several case reports have mentioned amyloid infiltration in bone marrow aspirates of patients with symptomatic multiple myeloma or systemic amyloidosis. (1) reported to detect amyloid in bone marrow smears in 40% of patients diagnosed with symptomatic multiple myeloma in the absence of symptomatic light-chain (AL) amyloidosis. We have occasionally noted amorphous material when analysing bone marrow smears on conventional cytology of patients with AL amyloidosis. With this project, we want to assess sensitivity to detect amyloid in bone marrow aspirates of patients with symptomatic AL amyloidosis with conventional bone marrow cytology and congored (CR) staining. Sensitivity was compared to bone marrow histology and fat aspiration. Furthermore, (2) reported a high incidence of hepatic and renal involvement for patients with interstitial amyloid deposits on bone marrow histology. Therefore, a correlation with organ involvement patterns was also performed.

MATERIALS & METHODS: Prospective analysis of bone marrow aspirates was performed at our amyloidosis outpatient clinic for patients with the diagnosis of systemic AL amyloidosis between October 2013 and February 2016. For 130 patients three bone marrow smears were stained with Pappenheim. At least one smear was stained with CR according to (3). The CR stained slides were either newly produced or represented slides primarily stained with Pappenheim left overnight in methanol to decolor. Samples were analysed with a 100 watt polarisation microscope (Zeiss Axioplan 2). For organ involvement, we utilised the current consensus criteria (Tours).

RESULTS: We analysed bone marrow aspirates and positively detected amyloid with CR staining in 46 cases for sensitivity of 35%. Amyloid was detected diffusely in 30 cases and localized in 16 cases. Diffuse infiltration either gave the impression of a “starry night” under polarised light or showed large clumps of amyloid. All diffuse cases showed vast areas of cloudy material on Pappenheim staining on usually all three and at least on one slide. Therefore, conventional bone marrow cytology had a sensitivity of 23% (30/130). The 16 localized cases occasionally seemed to be associated with blood vessels.

For 100 of the 130 patients 102 bone marrow histology results were available. Amyloid was detected in 61% (62/102). In two cases we found localized amyloid deposits when bone marrow biopsy was negative and two patients with diffuse amyloid infiltrates were not detected on histology.

For 107 of the 130 patients, we also analysed a subcutaneous fat aspirate to screen for amyloid. We positively detected amyloid in 97% (104/107) of the cases. Only one patient who was not detected on fat aspiration showed diffuse amyloid deposits on bone marrow aspiration.

Patients with diffuse amyloid infiltrates on bone marrow cytology significantly more often had hepatic (67% vs. 9%, p<0.001) and renal involvement (83% vs. 45%, p<0.001); and significantly less often cardiac involvement (37% vs. 69%, p<0.003) compared to patients without diffused amyloid infiltrates.

DISCUSSION & CONCLUSIONS: Amyloid infiltrates could be detected on conventional cytology in approximately 23% of patients with systemic AL amyloidosis. CR staining of bone marrow smears cannot be recommended as a screening tool for systemic AL amyloidosis as its overall sensitivity with 35% was far inferior to bone marrow histology with 61% and abdominal fat aspiration with 97%. The technique should rather be used when detecting such infiltrates in a patient with MGUS or MM as these slides can be stained with CR to establish the diagnosis of AL amyloidosis. Finally, when detecting vast amounts of amyloid on bone marrow slides AL amyloidosis with hepatic and renal involvement should be considered.

PB118

HOVON 104; A multicenter, prospective study of bortezomib and dexamethasone as induction treatment followed by high dose melphalan and autologous stem cell transplantation in patients with de novo amyloid light chain (AL) amyloidosis

MC Minnema1, K Nasserinejad2, B Hazenberg3, U Hegenbart4, L Noens5, P Ypma6, S Zweegman7, L Tick8, G Bos9, H Koene10, N Thuss2, P Sonneveld11, S Schönland12

1UMC Utrecht Cancer Center, Utrecht, 2Erasmus MC Cancer Institute-Clinical Trial Center, Rotterdam, 3UMCG, Groningen, 4Amyloidosis Center, University of Heidelberg, Heidelberg, 5UZ Gent, 6Haga Hospitals, Den Haag, 7VU University Medical Center Amsterdam, 8Maxima Medical Center, Eindhoven, 9University Hospital, Maastricht, 10St Antonius Hospital, Nieuwegein, 11ErasmusMC, Rotterdam. M.C.Minnema@umcutrecht.nl

Introduction
The HOVON 104 multicenter trial started in January 2012 as a successor of the HOVON 41 trial, in which we prospectively demonstrated the efficacy of the 2-step approach consisting of induction treatment followed by high dose melphalan (HDM) and autologous SCT (ASCT) (Haematologica 2015; 677, BJH 2004,543)

Rationale and study objectives
The aim is to investigate the efficacy and safety of induction treatment consisting of 4 cycles of bortezomib and dexamethasone (BD) followed by HDM and ASCT to improve the complete hematological response rate (CHR). Using induction therapy the treatment related mortality of the ASCT is < 5% and the hematological response (HR) is long-lasting. Since HR rate is associated with survival, a better response rate will translate into better overall survival.

Design
The study started as a phase III trial with a randomized design in the induction treatment (1:2), but due to slow accrual the dexamethasone induction arm was closed after including 23 patients, 7 in the dexamethasone arm and 16 in the bortezomib-dexamethasone (BD) arm. Bortezomib was given sc 1.3 mg/m2 twice a week for 2 weeks in a 21-day cycle, dexamethasone 20 mg orally on bortezomib days and the day thereafter. Stem cell mobilization was performed with GCSF only. HDM was administered in a total dose of 200 mg/m2 in 2 days, in case of GFR < 40 ml/min a dose reduction of 50% was given.

Statistical plan
The primary objective of the trial is to evaluate the efficacy of BD induction treatment followed by HDM and ASCT. The success rate is defined as the proportion of patients with a CHR at 6 months after HDM and auto-SCT. The required sample size is calculated based on the assumption that a CHR rate of 30% would be too low, while a CHR rate of at least 50% would be sufficiently high to further investigate this regimen. With an 80% power 44 eligible patients are needed. To take into account the potential dropouts due to ineligibility, 50 patients were registered.

Major in- and exclusion criteria
Inclusion and exclusion criteria are installed both at inclusion and before stem cell mobilization. Criteria included age between 18-70 years, a measurable plasma cell dyscrasia defined as a detectable M-protein with serum electrophoresis and/or level of involved FLC > 50 mg/L, a life expectancy > 3 months, WHO performance status 0-2, NYHA stage 1-2. Major exclusion criteria are Multiple Myeloma stage II and III, symptomatic orthostatic hypotension, cardiac ejection fraction < 45%, NT proBNP level > 5000 pg/ml and Troponin > 2 times ULN, Bilirubin > 2x ULN, GFR < 30 ml/min, NCI CTCAE grade peripheral sensory neuropathy > grade 2 or > gr 1 with pain.

Conclusion
The prospective, multicenter HOVON 104 phase II trial was closed for inclusion on the first of April 2016 because the target number was reached. The first results are expected by the end of 2016. This trial will provide information in a multicenter setting on the safety and efficacy of 4 cycles of BD, the percentage of patients that proceed to HDM and ASCT, the CHR rate and the PFS and OS of intensive treatment in de novo AL amyloidosis treatment using a 2-step approach.
Quantitation of tissue amyloid by novel Magnetic Resonance Imaging T2 relaxation sequences

N Hua1, JE Ward2, V Shibad2, G Yee1, LH Connors2, H Jara3, DC Seldin2, JA Hamilton1

1Department of Physiology and Biophysics, 2Alan and Sandra Gerry Amyloidosis Research Laboratory in the Amyloidosis Center, 3Department of Radiology, Boston University School of Medicine and Boston Medical Center, Boston, USA.

INTRODUCTION: Imaging the locations and amount of amyloid in the body remains a clinical challenge in systemic amyloidosis. An accurate assessment of tissue amyloid deposition would complement current diagnostic methods, such as echocardiography and endomyocardial biopsy, and provide a non-invasive means to monitor disease progression and therapeutic responses. In magnetic resonance imaging (MRI), T2 is a property of nuclear spin which varies with tissue type and disease stage. Here, we explore the potential of using T2 relaxation MRI to assess the amount of amyloid in ex vivo tissues from cases of AL amyloidosis.

MATERIAL & METHODS: Human myocardial specimens from 7 AL patients and 2 controls (CT) were studied. Frozen specimens stored at -20°C were thawed to room temperature, and imaged at 25°C using a Bruker 11.7T Avance instrument and a 20 mm birdcage coil. A spin echo sequence was applied: FOV=15mm², matrix size=64x64, slice thickness=1.25mm, TR=4000ms, echo spacing=6.4ms, echo=32, NEX=32. Signals were analyzed by monoexponential modelling using a least-squares algorithm to calculate T2. The T2 distribution pattern was visualized by histogram and mathematically assessed by skewness (the closer to 0, the more symmetric). Specimens were classified into 3 categories based on Congo red (CR) staining: 0, no amyloid (control); 1+, focal amyloid deposits; 2+, diffused interstitial amyloid deposition. Data are presented as mean ± SD; statistical significance is considered as p < 0.05.

RESULTS: A representative T2 map from an amyloid sample (dark band surrounded by light grey buffer) is shown in Fig 1a. In samples with amyloid deposits, the mean T2 was 44.0±2.8 ms, which was comparable to controls (T2=52.3±6.1 ms, p=0.21; however, the T2 histograms revealed distinct differences between AL and controls. In particular, 2/2 (100%) controls showed a single peak and symmetric (skewness=0.12±0.05) distribution of T2 values (Figure 1b, blue traces). In contrast, 6/7 (86%) of amyloid samples showed a binominal (2 peaks) distribution and skewed (skewness=0.66±0.41) T2 distribution (Figure 1b, red traces). Moreover, in the amyloid samples, skewness corresponded to amyloid amount as determined histologically; no amyloid (CR 0) had skewness=0.12±0.05 (n= 2), focal deposits (CR 1+) had skewness=0.27±0.04 (n=2), and diffused amyloid (CR 2+) had skewness=0.87±0.31 (n=5).

DISCUSSION & CONCLUSIONS: The asymmetry of T2 distribution differs in tissues with varying amounts of amyloid deposition and appears to be directly related to amyloid tissue burden. The MRI sequences may be useful in the clinic setting as a rapid, non-invasive and quantitative measurement of tissue amyloid for assessments of organ involvement, progression and response to treatment.


This work was funded by the Boston University Amyloidosis Research Fund.
Histological characterization of localized amyloidosis tissues

HL Cui1,2, LH Connors1,2, T Prokaeva1, M Skinner1, CJ O’Hara1,2

1Alan and Sandra Gerry Amyloidosis Research Laboratory in the Amyloidosis Center, 2Department of Pathology and Laboratory Medicine, Boston University School of Medicine and Boston Medical Center, Boston, MA USA

hcui@bu.edu

INTRODUCTION: Localized amyloidosis is a diagnosis based on the identification of amyloid deposits in only one tissue or organ site. While the much more frequently occurring systemic forms of amyloidosis feature amyloid infiltration at multiple and varied locations within vital organs, localized amyloid deposits do not typically occur in heart, liver, kidney or nerves. Current information about localized amyloidosis is limited, but several groups have reported an association of amyloid deposits with multinucleated giant cells (MGCs).1,2 Reactions ranging from a few cells per amyloid-containing histologic section to readily abundant forms have been demonstrated, but the genesis and role of the MGCs remains unclear. This report summarizes our experience with an ever-increasing referral population of localized amyloid cases during a recent 7-year interval.

MATERIALS & METHODS: Specimens and data from cases of localized amyloidosis evaluated at Boston University Amyloidosis Center clinic between January 2009 and February 2016 were reviewed. All patients had a diagnosis of amyloid based on positive Congo red staining3 of a biopsy from the affected site. The identity of the amyloid deposited protein was determined by immunohistochemistry, immunogold electron microscopy, or mass spectral analysis. Hematoxylin-eosin and anti-CD68 IHC staining were performed on archived tissues.

RESULTS: From January 2009 to 2016, 183 (13%) of 1450 new patients were diagnosed with localized amyloidosis. The group consisted of 111 females (61%) and with a median age at diagnosis of 59 years (range, 19-83); the majority were Caucasian (88%). The histologic details of 57 cases are shown in Table 1; a representative case demonstrating amyloid deposits and MGCs is depicted in Figure 1.

DISCUSSION & CONCLUSION: Several groups have studied the histology of localized amyloid deposits in an effort to identify distinctive morphologic features that may shed some light on the pathogenesis of the disease and possible differences with the systemic forms. In our study, MGCs were observed in 61% (31/57) of the localized cases of amyloid. The MGCs were of the foreign body giant cell type. Positive staining with CD68 provided evidence that MGCs were of macrophage lineage. In most instances, the MGCs were closely aligned to and surrounded the amyloid deposits; however, we were not able to definitively confirm that amyloid deposits were contained within the multinucleated giant cells or single macrophages. This work was supported by the Boston University Amyloid Research Fund and the Young Family Amyloid Research Fund.


<table>
<thead>
<tr>
<th>Location</th>
<th>No. of Cases</th>
<th>Amyloid Burden (Congo red)</th>
<th>AL Subtype N(%)</th>
<th>Cases with MGCs N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LC-1</td>
<td>LC-2</td>
</tr>
<tr>
<td>Air way-Lung</td>
<td>10</td>
<td>1-3+</td>
<td>3 (30)</td>
<td>3 (30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8 (80)</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>4</td>
<td>2-3+</td>
<td>0 (0)</td>
<td>1 (25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 (50)</td>
<td></td>
</tr>
<tr>
<td>Digestive system</td>
<td>7</td>
<td>2-3+</td>
<td>5 (71)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2 (29)</td>
<td></td>
</tr>
<tr>
<td>Ear, Nose &amp; Throat</td>
<td>15</td>
<td>1-3+</td>
<td>1 (7)</td>
<td>4 (27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9 (60)</td>
<td></td>
</tr>
<tr>
<td>Eye</td>
<td>5</td>
<td>2-3+</td>
<td>1 (20)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 (80)</td>
<td></td>
</tr>
<tr>
<td>Genitourinary</td>
<td>10</td>
<td>1-3+</td>
<td>4 (40)</td>
<td>1 (10)</td>
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<td></td>
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<td>6 (60)</td>
<td></td>
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<tr>
<td>Skin</td>
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<td>1-3+</td>
<td>2 (40)</td>
<td>0 (0)</td>
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<td></td>
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<td>4 (80)</td>
<td></td>
</tr>
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<td>Thyroid</td>
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<tr>
<td>Total</td>
<td>57</td>
<td>16 (28)</td>
<td>9 (16)</td>
<td>35 (61)</td>
</tr>
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</table>

Table 1. Histological Findings in 57 cases.

Figure 1. Amyloid deposits with MGCs in lung tissue. Sections stained with A. Congo red, B. Congo red polarized view, C. hematoxylin-eosin, and D. CD68. (400X)
Regulated expression of amyloidogenic immunoglobulin light chains in mice.

M Nuvolone1,2, S Sorce3, P Pelczar3, E Rushing1, F Lavatelli2, P. Rognoni2, G Palladini2, G Merlini2 and A Aguzzi1.

1Institute of Neuropathology, University Hospital of Zurich, Zurich, Switzerland. 2Amyloidosis Research and Treatment Center, Foundation Scientific Institute San Matteo, Department of Molecular Medicine, University of Pavia, Pavia, Italy. 3Institute of Laboratory Animal Science, University of Zurich, Zurich, Switzerland.

mario.nuvolone@usz.ch

INTRODUCTION:
In immunoglobulin light chain (AL) amyloidosis, clinical and experimental observations converge towards a direct toxic role of the amyloidogenic light chain (aLC) precursor. However, the molecular underpinnings of AL amyloidosis remain largely obscure, partly due to the paucity of preclinical models for this disease. Generation of conventional transgenic mice with constitutive overexpression of human aLCs is technically hampered by the potential toxicity of these proteins, possibly resulting in embryolethality and selection against high expressor lines1,2. To overcome these limitations, we have employed the Cre/loxP system to generate a conditional transgenic mouse allowing regulated expression an aLC.

MATERIAL & METHODS:
The cDNA encoding a human aLC (λ), termed MAB, was sub-cloned in the pCAG-CAT-Oligo vector3. The resulting expression vector consisted of (5’→3‘): a ubiquitous promoter (CAG), a reporter gene-stop cassette (CAT) flanked by two equally oriented loxP sites and the aLC (MAB). Transgenic mice obtained by pronuclear injection in C57BL/6J fertilized oocytes (termed CAG-CAT-MAB) were crossed with either mice expressing a constitutively active cre recombinase under the albumin promoter (Alb-cre), or with mice expressing a tamoxifen-inducible cre recombinase under the CAG-promoter (CAGGS-creER™). Expression of human aLCs was analyzed by RT-PCR, immunohistochemistry, Western blotting and ELISA.

RESULTS:
Pronuclear injection of the CAG-CAT-MAB vector resulted in the generation of three transgenic lines (line 179 to 181). CAG-CAT-MAB+/+ mice showed ubiquitous expression of the CAT reporter gene, but no expression of aLC, as expected. Crossing with Alb-cre transgenic mice resulted in hepatocyte-restricted expression of aLC, whereas crossing with CAGGS-creER™ transgenic mice lead to ubiquitous expression of aLC after tamoxifen administration. In both cases, human aLC could be detected in serum, with mice with ubiquitous expression showing higher levels than mice with hepatocyte-restricted expression. Mice with hepatocyte-restricted expression of aLC have been monitored for up to two years, with no evidence of amyloid formation. Mice with ubiquitous expression of aLC are in follow-up for the identification of amyloid deposits and phenotypic abnormalities.

DISCUSSION & CONCLUSIONS:
We have generated a novel transgenic mouse in which the expression of aLC can be activated in a spatially and temporally regulated manner. In mice with hepatocyte-restricted expression of aLC, all target organs (except the liver) are exposed to aLC exclusively coming from the circulation, resembling the clinical situation. Ubiquitous expression of aLC results in higher levels of circulating aLC. Additional cre transgenic lines can be used to further manipulate the temporal and spatial pattern of aLC expression.

This newly developed conditional transgenic mouse could prove instrumental to deepen our current mechanistic understanding of AL amyloidosis.

REFERENCES:
Incomplete DJH rearrangements of the IgH gene are frequent in AL Amyloidosis as compared with multiple myeloma and MGUS Patients, implying an earlier precursor differentiation.

T Sheikh¹, M Pick¹, E Slyusarevsky¹, G Pogrebijski¹, S Krichevsky ¹, D Ben Yehuda ¹, ME Gatt¹

¹ Department of Hematology, Hadassah Hebrew University Medical Center, Jerusalem, Israel.

rmoshg@hadassah.org.il

Introduction: Light chain amyloidosis (AL) results secondary to an underlying plasma cell monoclonal gammopathy. Dissimilar to multiple myeloma (MM) and monoclonal gammopathy of unknown significance (MGUS), AL patients have diverse chromosomal aberrations, implying that AL has a different genetic background than other plasma cell dyscrasias (PCD). During B-cell differentiation the V, D and J gene segments of the Ig gene locus are rearranged in an ordered fashion for the generation of the primary Ig repertoire. Ig heavy-chain (IGH), D to JH joining precedes V to DJH joining. After a functional complete VH rearrangement, the other incomplete DJH rearrangement IGH allele is generally excluded. Presence of an incomplete DJH rearrangement, therefore implies it occurring during earlier B-cell development at the pro-B stage. We hypothesized that AL will harbor more incomplete DJH rearrangements than MM or MGUS substantiating its different genetic differentiation basis of an earlier clonal B-cell disease.

Methods: BM aspirates of 334 PCD diagnosed patients (74 AL, 185 MM and 75 MGUS), routinely processed for complete and incomplete IGH rearrangements were compared. DNA Samples were correlated with disease features and clinical characteristics.

Results: Baseline characteristics differed among the three patient groups (MM, MGUS, AL), MM patients slightly younger, and M-protein isotype diverse (IgG more abundant in MM, IgM in MGUS and light chain-only / λ subtype in AL). None of these baseline variances significantly correlated with the IGH rearrangement type. 45.5%, of patients were found to harbor a complete VH, 29.3% an incomplete DJH, and 21.5% no IgH rearrangements were found. A substantial difference in the frequency of the rearrangement type among the groups was detected as shown in table below with AL having significantly more incomplete DJH rearrangements to all other patient groups as hypothesized (p < 0.007). There was no correlation found between the IGH rearrangement, or a specific VH or DJH subtype, nor with other clinical parameters, such as type of organ involvement, disease stage and prognosis.

Conclusion: Incomplete DJH rearrangements were detected in AL significantly more than in MM or MGUS patients, thus implying that AL has a different onco-genetic differentiation-origin background, as an earlier B-cell disease.

<table>
<thead>
<tr>
<th>Rearrangement Type</th>
<th>MGUS (N, %)</th>
<th>MM (N, %)</th>
<th>AL (N, %)</th>
<th>All (N, %)</th>
<th>p-Value</th>
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<td>Complete VH</td>
<td>26 (74.3)</td>
<td>103 (62.4)</td>
<td>23 (46)</td>
<td>152 (60.8)</td>
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<tr>
<td>Incomplete DH</td>
<td>9 (25.7)</td>
<td>62 (37.6)</td>
<td><strong>27 (54)</strong></td>
<td>98 (39.2)</td>
<td><strong>0.007</strong></td>
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POSTER PRESENTATION

PC1 – PC105
| PC1 | Immunoparesis Status In AL Amyloidosis At Diagnosis Affects Response And Survival By Regimen Type |
| PC2 | Immunoparesis In Newly Diagnosed AL Amyloidosis Is A Marker For Response And Survival |
| PC3 | Idelalisib For IgM-Associated AL Amyloidosis |
| PC4 | Cardiotoxicity Of Lambda Light Chains: Some Clinical And Epidemiological Aspects |
| PC5 | Potential Novel Biomarkers For Diagnostics Of AL Amyloidosis |
| PC6 | Progressive Or Refractory AL Amyloidosis Patients Are Particularly Responsive To The Addition Of Clarithromycin To An Imid Based Therapy. |
| PC7 | Free Light Chain Monomer-Dimer Patterns May Assist In Differentiation Of AL Amyloidosis From Benign Forms Of Plasma Cell Dyscrasia |
| PC8 | The Impact Of AL Amyloidosis On Absenteeism, |
| PC9 | The Relative Burden Of AL Amyloidosis On health-related quality of life in AL amyloidosis |
| PC10 | Treatment History, Tolerability And Impact On |
| PC11 | Psychometric Validation Of The SF-36V2® Health Survey |
| PC12 | Recruitment Of Light Chains By Homologous And Heterologous Fibrils Shows Distinctive Kinetic And Conformational Specificity. |
| PC13 | Interim Analysis Of Phase I Study Of Chimeric Fibril-Reactive Monoclonal Antibody 11-1F4 In Patients With AL Amyloidosis |
| PC14 | Improved Survival In AL Amyloidosis: A Population Based Study On 1,492 Patients Diagnosed In Sweden 1990-2013 |
| PC15 | AL Amyloidosis: Real World Evidence From Argentina |
| PC16 | The Effect Of Bone Marrow Plasma Cell Burden On Survival In Patients With AL Amyloidosis Undergoing High Dose Melphalan And Autologous Stem Cell Transplantation |
| PC17 | Phase 3 Study Of The Oral Proteasome Inhibitor Ixazomib For Relapsed/Refractory AL Amyloidosis: TOURMALINE-AL1 |
| PC18 | Characterization Of Patients With Predominantly Intramural Coronary Amyloidosis |
| PC19 | Left Ventricular Assist Device (LVAD) For Cardiogenic Shock In A Rare Case Of Intracoronary Light Chain Amyloidosis |
| PC20 | AL Amyloidosis (ALA) As Relapse Of Symptomatic Multiple Myeloma (MM): Dual Clinical And Biological Features And Outcome |
| PC21 | Patterns Of AL Amyloid Neuropathies Associated With Monoclonal IgM Gammopathy |
| PC22 | Neod001 Specifically Binds Aggregated Light Chain Infiltrates In Multiple Organs Of Patients With AL Amyloidosis And Promotes Phagocytic Clearance Of Light Chain Aggregates In Vitro |
| PC23 | A Multifactorial Disease Requires A Multidisciplinary Approach: An Investigation Into Immunoglobulin Light Chain Amyloidosis Using A Combination Of Computational, Cell And Biochemical Techniques. |
| PC24 | Prospective Evaluation Of The Clinical And Prognostic Implications Of Blood Pressure Monitoring And Baroreceptor Reflex Sensitivity (Brs) In Patients With AL Amyloidosis |
| PC25 | Hematologic And Renal Improvement Of Monoclonal Immunoglobulin Deposition Disease After Treatment With Bortezomib-Based Regimens |
| PC26 | Bortezomib Before, In And After Autologous Stem Cell Transplantation In Patients With Newly Diagnosed AL Amyloidosis |
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Immunoparesis status in AL amyloidosis at diagnosis affects response and survival by regimen type

E Muchtar1, A Dispenzieri1, SK. Kumar1, FK Buadi1, MQ Lacy1, S Zeldenrust1, SR Hayman1, N Leung1, R Chakraborty1, S Russell1, D Dingli1, JA Lust1, Y Lin1, Pt Kapoor1, R Go1, RA Kyle1, VS Rajkumar1 and MA Gertz1

1 Division of Hematology, Mayo Clinic, Rochester, Minnesota 2 Hospitalist Services, Essentia Health St. Joseph’s Hospital, Brainerd, Minnesota 3 Division of Hematopathology, Mayo Clinic, Rochester, Minnesota

INTRODUCTION: Clinical tools to guide in the selection of appropriate treatment in AL amyloidosis are not well developed.

MATERIAL & METHODS: We evaluated the response and outcome for various regimens at first line treatment (n=681) and first progression (n=240) stratified by the immunoparesis status at diagnosis. Immunoparesis was assessed by the average relative difference (ARD) of the uninvolved immunoglobulins, classifies patients into a negative ARD (i.e. significant immunoparesis) or a positive ARD (i.e. no or modest immunoparesis). Treatment was categorized as autologous stem cell transplant (ASCT) and four non-transplant regimens (melphalan-based; bortezomib-based, Immunomodulatory drug (IMiD)-based and dexamethasone alone).

RESULTS: Patients with significant immunoparesis had a significantly worse ≥VGPR (58%), PFS (median 30 months) and OS (108 months) compared to those without significant immunoparesis (80%, 127 months, median not reached, respectively; P<0.001 for all comparisons). Among the non-transplant regimens, melphalan resulted in an unfavorable PFS (11 vs 27 months; P<0.001) and OS (30 vs 74 months; P=0.001) in patients with significant immunoparesis compared to those without significant immunoparesis. In contrast, no significant difference in PFS and OS between immunoparesis groups was seen for those treated with bortezomib (PFS 14 vs 23 months, P=0.13; OS 57 vs 41 months, P=0.61) or IMiD (PFS 20 vs 13 months, P=0.84; OS 32 vs 16 months, P=0.4). At first progression, immunoparesis status did not impact response or survival to any regimen.

DISCUSSION & CONCLUSIONS: Melphalan at first line provided poorer outcomes for patients with significant immunoparesis, while bortezomib or IMiDs were more likely to overcome the adverse prognosis associated with significant immunoparesis.

Fig. 1: Overall survival stratified by the immunoparesis status at diagnosis and first line regimen type. (A) Autologous stem cell transplant (B) melphalan-based regimen (C) bortezomib-based regimen (D) Immunomodulatory-based regimen
Immunoparesis in newly diagnosed AL amyloidosis is a marker for response and survival


1 Division of Hematology, Mayo Clinic, Rochester, Minnesota 2 Hospitalist Services, Essentia Health St. Joseph’s Hospital, Brainerd, Minnesota 3 Division of Hematopathology, Mayo Clinic, Rochester, Minnesota

INTRODUCTION: Immunoparesis is an adverse prognostic marker in plasma cell proliferative disorders. Its impact in AL amyloidosis has not been explored in depth.

MATERIAL & METHODS: Newly diagnosed AL amyloidosis patients (n=998) were evaluated for immunoparesis by two methods. The first method was qualitative, considering the number of suppressed uninvolved immunoglobulins below the lower limit of normal (LLN) (none, partial, all). The second method was quantitative, assessing the average relative difference (ARD) of the uninvolved immunoglobulins from the LLN. Patients with a positive ARD were considered those with no or modest immunoparesis whereas a negative ARD was considered significant immunoparesis.

RESULTS: By the qualitative method, 28% of patients had no suppression of the uninvolved immunoglobulins, 49% had partial suppression and 23% had suppression of all the uninvolved immunoglobulins. Patients with suppression of all the uninvolved immunoglobulins were less likely to achieve VGPR or better to first line treatment (44%) compared to patients with partial suppression (68%) or preserved uninvolved immunoglobulins (64%; p<.0001). Additionally, patients with suppression of all the uninvolved immunoglobulins had a shorter survival compared to the respective comparators (median 18 vs. 54 vs. 52 months; p<.0001). In the quantitative method, 64% of the patients had a positive ARD values, whereas 36% of patients had a negative ARD. Patients with negative ARD values were less likely to achieve VGPR or better (48%) and had a shorter survival (median 24 months) compared to patients with positive ARD (69%, 57 months, respectively; p<.0001). In a multivariate analysis for survival, including age, dFLC, Mayo stage, stem cell transplant and either method of immunoparesis assessment, both methods retained an independent impact.

DISCUSSION & CONCLUSIONS: Significant immunoparesis has a negative impact on response and survival in newly diagnosed AL amyloidosis. There is a better discrimination of outcomes for the quantitative assessment as compared with the qualitative assessment. The prognostic value of the uninvolved immunoglobulins is strengthened by the widespread availability of immunoglobulin measurements and its high reproducibility.

Fig. 1: Overall survival curves using the Kaplan-Meier method.

(A) by the qualitative method (B) by the quantitative method
Idelalisib for IgM-associated AL amyloidosis

S Sarosiek¹, V Sanchorawala¹, A Shelton¹, C Varga¹, JM Sloan¹

¹Amyloidosis Center, Boston University School of Medicine and Boston Medical Center; Boston, MA USA.

Shayna.Sarosiek@bmc.org

INTRODUCTION: AL amyloidosis with an associated IgM paraprotein accounts for approximately 4-6% of all AL amyloidosis diagnoses. Unlike the majority of AL amyloidosis patients who have an underlying monoclonal plasma cell disorder, AL patients with an IgM paraprotein often have a clonal population of lymphoplasmacytic or lymphoid cells. Due to the rarity of this subgroup of AL amyloidosis patients, there are limited clinical data and no standard treatment for this patient population.

Historically, patients with IgM-associated AL amyloidosis have been treated with standard AL amyloidosis treatments, such as high-dose melphalan and bortezomib-based therapies. There are retrospective data to support the use of these treatments. In recent years, the therapy has been adapted to incorporate rituximab, as a single agent or in combination therapy, in an effort to target the clonal CD20+ lymphoid or lymphoplasmacytic cells. Hematologic responses have been reported, but toxicities are not minimal, particularly in the combination therapies that produce significant hematologic responses. At this time there continues to be a need for safe and effective treatment options for IgM-associated AL amyloidosis, as relapses and treatment toxicity continue to be issues in this patient population.

Idelalisib is a first-in-class oral inhibitor of the delta isoform of phosphatidylinositol-3-kinase (PI3Kδ) which targets signal transduction downstream of the B-cell receptor in malignant B cells. It was recently approved for the treatment of relapsed or refractory chronic lymphocytic lymphoma, small lymphocytic lymphoma and follicular lymphoma. There are also early data reporting favorable and durable response rates when used as monotherapy in patients with Waldenstrom’s macroglobulinemia. Based on these experiences, a Phase II clinical trial was proposed using idelalisib for treatment of IgM-associated AL amyloidosis with the intention of finding a well-tolerated treatment targeting the monoclonal lymphoid or lymphoplasmacytic cells.

METHODS: In this study, eligible patients with relapsed IgM-associated AL amyloidosis are being enrolled and will receive treatment with idelalisib at the standard dose of 150mg twice daily. To be eligible, patients must have biopsy-proven AL amyloidosis with an IgM paraprotein identified on serum immunofixation electrophoresis or light chain-restricted CD20+ lymphoplasmacytic population on biopsy of bone marrow or lymph node. Additional criteria require that the patients be at least 18 years old, with an ECOG status ≤2, a difference in serum free light chains of >30 mg/L or IgM paraprotein >0.5g/L, and adequate bone marrow function (absolute neutrophil count ≥1,000/mm³ and platelets ≥50,000/mm³). Exclusion criteria include, but are not limited to, previous treatment with idelalisib, compromised end-organ function, other active malignancy, lytic bone lesions, t(11,14) identified on bone marrow, or other amyloid-directed therapy within 28 days. All participants will be maintained on prophylaxis to prevent Pneumocystis jirovecii infection, have monthly monitoring for CMV reactivation and fungal infection, and have close monitoring of complete blood counts. Participants will continue therapy with idelalisib until progression, unacceptable toxicity, or decision to withdraw from the trial. The primary end point is the hematologic overall response to treatment with idelalisib. Secondary end points include progression free survival, organ response, safety and tolerability, and quality of life impact. The accrual goal for the trial is 18 patients.


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**Cardiotoxicity of lambda light chains: some clinical and epidemiological aspects**

M Di Girolamo\(^1\), MT Petrucci\(^2\), M Nowakowski\(^3\), A Tranghese\(^4\)

\(^1\)Department of Medicine, AFaR – “San Giovanni Calibita” Fatebenefratelli Hospital, Rome, Italy \(^2\)Department of Cellular Biotechnologies and Haematology “Sapienza” University of Rome, Italy. \(^3\)Department of Medicine and \(^4\)Cardiology General Hospital, San Giovanni di Dio” Melfi, Italy

**INTRODUCTION** The mechanisms involved in the formation and organ-specific tropism of light chains amyloid fibrils remain unclear, related to structure of monoclonal immunoglobulin light chain, and probably for some “homing conditions” favoring some damaging effects of the fibrils on target organ.

**MATERIAL & METHODS** A retrospective study was conducted on two groups of patients with AL amyloidosis in order to evaluate the differences of cardiac involvement as prognostic factor:

Group A 85 pts [M 54, F 31 (range 43-86 yrs)] with “primary” AL amyloidosis, a multi-organ disease due to the deposition of Ig monoclonal light chains (LC) commonly occurring with a nephrotic syndrome associated or not to a cardiac involvement, leading to congestive heart failure. Group B 23 pts [M 15, F 8 (range 41-80 yrs)] with “secondary” AL amyloidosis: an uncommonly feature, approximately 15% of the pts with multiple myeloma (MM), developing at the diagnosis of MM or later, during its clinical course, with symptomatic organs failure due to fibrils LC deposition.

**RESULTS** The light chain (LC) restriction involved in the two groups were similar: Group A [LC \(\lambda = 60\) (70.5%) LC \(\kappa = 25\) (29.5%)]; Group B [LC \(\lambda = 15\) (65.2%) LC \(\kappa = 8\) (34.8%)]. The bone marrow % plasma cells at the diagnosis were: Group A 10.4% (range 4-28); Group B: 36.4% (range 15-70)

**DISCUSSION** Most frequently the group A pts had kidney involvement (78%) followed by heart (65.8%) while (group B pts) had the heart (87.6%) followed by kidney (70.8%). An interesting data (table 1) consist in the timing of cardiac amyloid involvement in group B: that was present only in 9/23 pts (39%) at onset of MM but several months later (range 9-17) because of cardiac failure in progress, (proven by a combination of clinical, echocardiography and, in two cases, by myocardial biopsy) in 20/23 pts (87%). This phenomenon was more evident among the pts having a LC \(\lambda\) restriction: a progressive increasing cardiac failure involving the majority of them, from 20% (3/15) at the diagnosis of MM up to 93% (14/15), many months after starting therapy for MM. That was not observed in the pts with LC \(\kappa\) restriction [at onset 75% (6/23) -> at follow up 75% (6/23)]. Although cardiac death in AL amyloidosis is usually associated with extensive myocardial infiltration, this phenomenon does not appear to correlate with the degree of heart failure or survival. Some “cardiotoxic” LC proteins, mainly LC \(\lambda\), could contribute to the rapid progression of cardiomyocyte dysfunction, independently of their deposition, inducing e.g. cardiomyocyte oxidant stress and some alterations in cellular redox status.

**CONCLUSIONS** Our preliminary data, if confirmed in larger studies, could suggest that cardiac homing in multiple myeloma could be modified by an amyloidogenic factor quickly favoring pathological fibrillar deposits (especially of the LC \(\lambda\)) in the myocardium (e.g. some chemotherapy drugs used for myeloma).

In our opinion it is therefore advisable that in the MM follow up, especially if present a LC\(\lambda\), should be augmented the surveillance on the risk of cardiac involvement for some reasons: therapeutic choices in drugs with lower cardiotoxic effects, decisional timing on marrow transplantation, early recognition of symptoms of the heart involvement, improving research in to new strategies aimed to eliminate circulating LC proteins or protecting cardiomyocytes with new antioxidants substances.

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Table 1. Heart and kidney involvement in primary and secondary AL pts according to the light chain type

| Light chains \( \kappa \) (n = 25) | 17 (68%) | 15 (60%) | 19 (76%) | 19 (76%) |
| Light chains \( \lambda \) (n = 60) | 45 (75%) | 46 (76.6%) | 47 (78.3%) | 48 (80%) |
| n = 85 | Heart | Kidney | Heart | Kidney |

| Light chains \( \kappa \) (n = 8 ) | 6 (75%) | 4 (50%) | 6 (75%) | 6 (75%) |
| Light chains \( \lambda \) (n = 15) | 3 (20%) | 8 (53.3%) | 14 (93.3%) | 10 (75%) |
| n = 23 | Heart | Kidney | Heart | Kidney |

| 9 pts | 12 pts | 20 pts | 16 pts |
Potential novel biomarkers for diagnostics of AL amyloidosis

Z Kufova1,2,5, E Kryukova1,2, L Brozova3, L Besse4, K Growkova1,2,5, L Sedlarikova6, J Filipova1,2,5, J Nekvindova1, T Sevcikova1,2,5, S Grosicki9, A Jurczyszyn9, J Jarkovsky3, S Sevcikova6, L Zahradova1, F Kryukov1,2, R Hajek1,2,6

1 Blood Cancer Research Group, Department of Hematooncology, Faculty Hospital Ostrava, Ostrava, Czech Republic. 2 Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic. 3 Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic. 4 Department of Hematology/Oncology, Cantonal Hospital St. Gallen, St Gallen, Switzerland. 5 Department of Biology and Ecology, Faculty of Science, University of Ostrava, Ostrava, Czech Republic. 6 Babak Myeloma Group, Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic. 7 Institute of Clinical Biochemistry and Diagnostics, University hospital Hradec Kralove, Czech Republic. 8 Department of Cancer Prevention, School of Public Health, Silesian Medical University in Katowice, Poland. 9 Jagiellonian University Medical College Department of Hematology, Krakow, Poland

zuzana.kufova@fno.cz

INTRODUCTION: Immunoglobulin light chain amyloidosis (ALA) is a plasma cell dyscrasia characterized by deposition of amyloid fibrils in various organs and tissues. Until now, no systematic study of transcriptome changes in ALA, neither miRNAs profile was evaluated in comparison with other monoclonal gammopathies. Our aim is to reveal specific novel biomarkers with ability to differentiate ALA from other monoclonal gammopathies.

MATERIAL & METHODS: Gene expression profiling data from GEO database (GSE6477, GSE24128) comprised of ND (n=15), MGUS (n=21), SMM (n=24), MM (n=69) and ALA (n=16) patients were used. To identify the ALA specific expression profile, we applied SAM algorithm. Genes with FC ≥1.5 or ≤0.5 and FDR <0.001 were considered as significant. MiRNAs profiling using TaqMan Low Density Arrays was used to find differentially expressed miRNAs among 15 ALA, 6 MGUS, 10 MM patients and 11 healthy donors (HD). Data normalization was performed using geometric mean of three most stably expressed miRNAs (miR-126, miR-24, miR-484).

RESULTS: Comparison of gene expression profile of ALA vs. ND, MGUS, SMM and MM revealed 256 unique genes being differentially expressed in ALA. Finally, 16 genes with the best predictive power have been discovered: RPS6, RPS14, RPS17, RPLA18A, TMEM66, EIF3L, CCDC72, GLTSCR2, NDUFS6, DUSP1, IGLJ3, IGHM, IGKV1-5, IGKC/IGKV1-5/IGKV4-1. Strikingly, those 16 genes as well as other elements from the set of 256 differentially expressed genes represent mostly ribosomal proteins and immunoglobulin regions.

Moreover, fifty miRNAs were differentially expressed in ALA compared to MM, MGUS and HD (p<0.05). Five of them were defined to have prognostic value: miR-21, miR-25, miR-328, miR-451, miR-134. In addition, serum miR-134, which was previously described as a marker of acute coronary syndrome, is present in ALA as well.

DISCUSSION & CONCLUSION: We assume that presented molecular signature has the best predictive power and allows us to detect specific aberrant clone responsible for pre-amyloid production and could in particular serve as baseline for further detailed study. We identified five serum miRNAs with a potential to distinguish ALA patients from MM, MGUS and healthy donors with high sensitivity and specificity. Moreover, we described increased level of miR-134 that could serve as biomarker of end-organ damage in ALA patients.

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Progressive or refractory AL Amyloidosis patients are particularly responsive to the addition of clarithromycin to an IMiD based therapy.

A Shaulov¹, C Ganel², N Benyamini³, N Goldschmidt¹, D Lavie¹, D Libster¹, A Guarl¹, B Avni¹, ME Gatt¹

¹ Department of Hematology, Hadassah Hebrew University Medical Center, Jerusalem, Israel. ² Department of Hematology, Share-Zedek Medical Center, Jerusalem, Israel ³ Department of Hematology, Rambam Medical Center, Haifa, Israel

rmoshg@hadassah.org.il

Introduction: Achievement of a significant hematologic response (HR) – i.e. very good partial response (VGPR) or complete response (CR), correlates with prolonged progression free (PFS) and overall survival (OS) in AL amyloidosis (AL) patients. Immune-modulators (IMiD) are effective in AL, used mostly for treatment of relapsed patients. Patients progressing on second line therapy have limited options for further treatment. Clarithromycin is an antibiotic with no therapeutic effect as a single agent in multiple myeloma (MM) patients. However, when combined with IMiDs, non-randomized reports are showing favorable results. We report the efficacy of clarithromycin addition to a cohort of AL patients already treated by IMiDs, progressing or having a non-sufficient response, thus necessitating a change in therapy.

Patients and Methods: Patients diagnosed 2008-2015 with AL or with light chain deposition disease (15 and two patients, respectively) and MM (26 patients), treated with second line or more IMiD based therapy. Clarithromycin was added to the protocol when patients had a non-sufficient response (i.e. sustained partial remission (PR), stable disease (SD) or progressive disease (PD)).

Results: Most AL patients had cardiac (82.4%) renal (58.8%), or multi-organ ≥ 2 (82.4%) involvement. Their median proBNP prior to treatment with clarithromycin was 4200 pg/ml (range 100-20000), 11 (64.7%) had a MAYO stage 3 cardiac disease, and (82.4%) were resistant to bortezomib. Most patients, 14 (82.4%), were treated with lenalidomide, three (17.7%) with thalidomide and one (5.9%) with pomalidomide. Before clarithromycin addition, 14 patients (82.4%) had a PD or SD and 3 (17.6%) a sustained PR (non-sufficient response). Response rate was remarkable: HR in 16 (94.1%) with VGPR in 6 (35.3%) and CR in 7 (41.2%) [figure]. At a median follow up of 11 months (1-42.2), PFS and OS has not been reached. In comparison to 26 MM patients treated similarly (not presented here), 11 (42.3%) achieved a HR, and not as deep or sustained responses. Six (35.3%) patients died (two while on treatment) of infection or disease related organ failure. Most adverse events (AE) were gastrointestinal and infectious, including 10 (58.8%) serious AE.

Conclusion: In a cohort of high risk and resistant AL patients, non-responsive or refractory to an IMiD-based therapy, the addition of clarithromycin resulted in a striking response rate, and a favorable outcome.

Response change

![](response_change.png)
Free light chain monomer-dimer patterns may assist in differentiation of AL amyloidosis from benign forms of plasma cell dyscrasia.

ME Gatt1, B Kaplan2, D Yoge3, E Slyusarevsky1, G Pogrebijski1, S Golderman2, O Kukuy3, A Livneh 2,4

1 Department of Hematology, Hadassah Hebrew University Medical Center, Jerusalem, Israel. 2 Heller Institute of Medical Research, Sheba Medical Center, Tel-Hashomer, Israel 3 Institute of Nephrology and Hypertension, Sheba Medical Center, Tel-Hashomer, Israel 4 Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

rmoshg@hadassah.org.il

Introduction: Plasma cell dyscrasias (PCD) comprise a spectrum of diseases, ranging between monoclonal gammopathy of unknown significance (MGUS) and AL amyloidosis (AL), and characterized by the presence in the serum of monoclonal immunoglobulins. The discrimination between benign forms (MGUS and smoldering myeloma (SMM)) and malignant forms (AL amyloidosis and multiple myeloma (MM)) is often difficult, as routine serum/urine tests for monoclonal protein are insufficient. Previously, we have shown that serum-free light chain (FLC) monomer-dimer (M-D) patterns may distinguish between malignant and benign types of PCD.

Aim: To evaluate the capacity of the FLC M-D analysis to distinguish between AL amyloidosis and benign PCD in high risk investigated patients with PCD.

Methods: Serum samples of patients with AL clinical suspicion, were analyzed in a blinded manner. Quantitative western blotting was used to estimate the relative quantity of FLC monomers (M) and dimers (D). Dimerization pattern (D/M ratio) and clonality indices (kappa/lambda ratio) were calculated to determine the type of the samples (benign or malignant). Degree of agreement between the conventional diagnosis (gold standard) and M-D analysis was evaluated.

Results: There were 108 samples, of which 56 were of patients with AL and 5 of patients with light chain deposition disease (LCDD) at diagnosis or under treatment (16 with complete remission (CR) and 4 with very good partial remission (VGPR)), and 47 of patients with a benign disease (MGUS (29), SMM (11) and localized AL (7)).

Of 39 samples from diagnosed AL patients with active disease, 93.3% (36) fulfilled the M-D criteria for diagnosis of AL. Of the 47 samples from patients with benign PCD, 29.8% (14) tested falsely positive by the M-D analysis. Thus, the sensitivity of the analysis was 92.3% (95%CI 79.1 to 98.3) and specificity 70.2% (95%CI 57.5 to 82.7); with a remarkable negative predictive value of 91.7%, and a positive predictive value of 72%. In addition, of the 20 patients in CR or VGPR, only one tested positive (and actually he had relapsed 3 months later).

Conclusion: Determination of FLC M-D patterns offers a highly effective tool in the diagnostic assessment of patients with PCD.
The impact of AL amyloidosis on absenteeism, reduced productivity, and job loss

S Guthrie1, MK White2, KL McCausland2, M Bayliss3

1 Prothena Biosciences Inc, South San Francisco, CA, USA. 2 Optum, Lincoln, RI, USA. spencer.guthrie@prothena.com

INTRODUCTION: Debilitating chronic conditions and their treatments often negatively impact patient’s ability to work, resulting in absenteeism, reduced productivity, and job loss. Light-chain (AL) amyloidosis is a rare disease in which misfolded light chains are deposited in tissues, which may lead to organ failure, disability, and death. Current treatments are known to affect patients’ functioning and well-being, but there is little evidence to date on the impact of AL amyloidosis on patients’ ability to work. The objective of this study is to describe the impact of AL amyloidosis on patients’ work using data from qualitative and quantitative research.

METHODS: Data for these analyses were collected from two phases of a broad research program on the experience of patients with AL amyloidosis. First, qualitative in-depth individual telephone interviews were conducted with 10 patients. Results are presented from coded interview transcripts that were analyzed using a grounded theory approach to identify themes.

Second, a quantitative online survey including a battery of patient-reported outcome measures was conducted in a separate sample of patients (n = 341). The data presented are based on the Work Productivity and Activity Impairment (WPAI) questionnaire for the subset of employed patients, including a single-item measure of the number of hours absent from work due to AL amyloidosis and a multi-item scale assessing overall lost productivity. Wilcoxon-Mann-Whitney tests were used to compare mean WPAI scores by time since diagnosis (< 12 months ago vs. ≥ 12 months ago) among those with cardiac involvement.

RESULTS: In qualitative interviews, seven of 10 patients reported that AL amyloidosis impacted their ability to work, manifesting as loss of focus or productivity, absenteeism including extended leaves of absence, and job loss. Patients reported underperforming at work and attributed this to symptoms, treatment side effects, and time required for doctor visits. Most felt their employers were supportive of their health needs; however, in some cases, job loss led to financial difficulties for families and frustration at subsequent changes in household roles.

In the quantitative study, 115 patients (38.3%) were currently employed. Of these, the mean age was 56.1, 56% were female, and 10% were non-Caucasian. On average, employed patients reported being absent from work five hours per week and a 27.6% reduction in overall work productivity due to AL amyloidosis. Patients with cardiac involvement reported significantly higher absenteeism compared to those without cardiac involvement (mean hours absent per week: 15 vs. 2.5 hours, respectively, p<0.02). Within the subgroup with cardiac involvement, overall lost work productivity for those diagnosed within the past year was twice that of patients with cardiac involvement who were diagnosed more than a year ago (54.4% vs. 25.4%, respectively, p<0.04).

DISCUSSION & CONCLUSIONS: These results indicate that AL amyloidosis has a significant impact on patients’ work, causing absenteeism, impaired productivity, and job loss. These results highlight additional costs of AL amyloidosis that are not related to medications or procedures, but are borne by patients, their employers, and their families. Advancements in treatment options for patients with AL amyloidosis and increased attention to patients’ functioning and well-being could potentially minimize these hidden costs.

Study supported by: Prothena Biosciences Inc
The relative burden of AL amyloidosis on health-related quality of life

M Bayliss¹, MK White¹, KL McCausland¹, S Guthrie²

¹ Optum, Lincoln, RI, USA. ² Prothena Biosciences Inc, South San Francisco, CA, USA.
spencer.guthrie@prothena.com

INTRODUCTION: The SF-36v2® Health Survey (SF-36v2) is a widely used general patient-reported outcome survey that can describe and quantify the impact of disease and its treatment on health-related quality of life (HRQoL). Light chain (AL) amyloidosis is a rare disease characterized by misfolded amyloid protein deposits in tissues and vital organs, and little is known about the burden of AL amyloidosis on HRQoL. The objective of this study is to compare the HRQoL profile of patients with AL amyloidosis and key patient subgroups to a general US population (USP) sample.

METHODS: The SF-36v2 was administered in an online, cross-sectional study of adults (≥ 18 years of age) with self-reported AL amyloidosis (n = 341). The SF-36v2 measures eight domain scales (physical functioning (PF), role limitations due to physical health problems (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role limitations due to emotional health problems (RE), and mental health (MH)) and two component summary measures: physical (PCS) and mental (MCS). Analysis of variance was used to compare the norm-based SF-36v2 scores from patients with AL amyloidosis to samples representing the USP. The USP sample size ranged from 4,024 – 4,036 for specific SF-36v2 scores. The USP data were adjusted to the age and gender distribution of the patient sample using separate ordinary least-squares regression models, with each SF-36v2 scale or summary score as a dependent variable. Using the same method, we evaluated the burden of two key subgroups: 1) patients with recent diagnosis (within the past year) (n = 52); and 2) patients with cardiac involvement (n = 178).

RESULTS: Relative to USP norms, HRQoL of patients with AL amyloidosis is significantly worse on scores from all eight SF-36v2 domain scales and both summary measures (p < 0.05 for all). The largest decrement was in GH, where the AL amyloidosis patient mean was a full standard deviation worse than the USP (39.3 vs. 49.0, respectively, Cohen's d -0.654; p < 0.001). Large decrements (more than a half standard deviation) also were seen in PF (24.5 vs. 36.9), RP (40.5 vs. 47.6), VT (44.5 vs. 50.2), SF (43.6 vs. 49.6), and PCS (40.7 vs. 46.7).

Subgroup analysis showed that, relative to the USP, patients (1) recently diagnosed with AL amyloidosis and (2) with cardiac involvement had large decrements in each of the SF-36v2 domain scales and both summary measures (p < 0.05 for all). The largest decrements for each subgroup, relative to USP, were:

1. Recently diagnosed: All scores except BP were at least a half standard deviation lower than USP, with three domain scores at least one standard deviation lower (RP, GH, SF).

2. Cardiac involvement: Four domain scores and one summary measure score were at least a half standard deviation lower than USP (PF, RP, GH, VT, and PCS).

DISCUSSION & CONCLUSIONS: Results indicate that AL amyloidosis patients have broad HRQoL deficits across all areas of physical and mental functioning compared to the general USP, with greater impact evident among key AL amyloidosis subgroups – in particular, patients diagnosed within the past 12 months. Understanding the burden of AL amyloidosis can help physicians identify ancillary treatments and services that may ease their burden and ultimately improve patients’ HRQoL.

Study supported by: Prothena Biosciences Inc
INTRODUCTION: Light-chain (AL) amyloidosis is a rare disease characterized by misfolded amyloid protein deposits in tissues and vital organs. There are currently no FDA- or EMA-approved medications indicated for AL amyloidosis; however, chemotherapy, stem cell transplants (SCT), and immunomodulatory drugs can reduce the production of amyloid-forming light chains. All existing regimens have tolerability problems due to treatment-related symptoms (TRSs). The SF-36v2® Health Survey (SF-36v2) is a widely-used general health-related quality of life (HRQoL) survey that can be used to describe and quantify the impact of many diseases and treatments. The objective of this study is to describe the history of treatments, past and current TRSs, and impact on HRQoL among a diverse sample of individuals with AL amyloidosis.

METHODS: We report baseline data from an online non-interventional study was initiated in 2015 among patients with self-reported AL amyloidosis (n = 341). Patients reported their current and prior treatment for AL amyloidosis. Aspects of TRSs were captured based on the following: 1) lifetime history of TRSs (dichotomous variable); 2) consequence of TRSs (discontinuation of a treatment; reduction of a treatment; or maintenance of treatment despite TRSs); and 3) ability to tolerate the current AL amyloidosis treatment (based on a 4 point scale from “extremely poorly” to “very well,” higher scores indicate better tolerability). The prevalence of each treatment type and TRSs were estimated. The patients’ ability to tolerate the current treatment was evaluated in relation to specific medications and HRQoL (as measured by the SF-36v2® Health Survey Physical [PCS] and Mental [MCS] Component Summary scores) using chi square tests for categorical variables and t-tests and ANOVA for continuous measures.

RESULTS: The most commonly reported treatments were dexamethasone (81% reported ever being treated, 52% reported as the current treatment), bortezomib (72% ever, 36% current), SCT (53% ever, 24% current), melphalan (47% ever, 15% current), cyclophosphamide (46% ever, 20% current), and lenalidomide (28% ever, 15% current). Many patients reported combination treatments, including cyclophosphamide+bortezomib+dexamethasone (CyBorD, 17% current). Half of the patients (51%) had received three or more different treatments. Nearly three-quarters (71%, n = 226) reported ever having problems tolerating AL amyloidosis treatment, of which nearly half (47%, n = 107) had discontinued at least one treatment. Nearly half (46%) of those currently being treated reported some tolerability issue (less than very good tolerability). Tolerability varied across the common treatments from a low of 3.22 (SD = 0.90) for cyclophosphamide to a high of 3.61 (SD = 0.52) for SCT. Problems with tolerating current medications corresponded with decrements in HRQoL (both MCS and PCS, p<0.001).

DISCUSSION & CONCLUSIONS: Lifetime history of TRSs was high. Discontinuation of life-saving AL amyloidosis treatments was fairly common, though most patients were able to tolerate their current regimen. The high prevalence of treatment discontinuation and history of multiple AL amyloidosis treatments suggests that physicians and patients try a variety of treatments to balance tolerability and efficacy. TRSs are associated with decrements in HRQoL, over and above the burden of AL amyloidosis. These findings highlight the importance of assessing HRQoL during treatment for AL amyloidosis to better understand tolerability, and the need for more treatment options for AL amyloidosis, particularly those with favorable tolerability.

Study supported by: Prothena Biosciences Inc
Psychometric validation of the SF-36v2® health survey in an AL amyloidosis population

MK White1, M Bayliss1, KL McCausland1, S Guthrie2

1 Optum, Lincoln, RI, USA. 2 Prothena Biosciences Inc, South San Francisco, CA, USA. spencer.guthrie@prothena.com

INTRODUCTION: Light chain AL amyloidosis, a rare protein misfolding disease, leads to deficits in health-related quality of life (HRQoL). Patients with AL amyloidosis present with a wide variety of non-specific symptoms, organ involvement, and functional impairment. The SF-36v2® Health Survey (SF-36v2), a general HRQoL survey, has been used to quantify the impact of AL amyloidosis on HRQoL, though to-date there is no evidence of its psychometric validity for use with AL amyloidosis patients. The objective of this study is to document the psychometric properties of the SF-36v2 among AL amyloidosis patients, including tests of data quality, scaling success, reliability, and validity.

METHODS: Adults (≥ 18 years old) with self-reported AL amyloidosis completed baseline (n = 341) and one-month follow-up (n = 252) surveys online to assess HRQoL, clinical and socio-demographic characteristics. Data quality evaluation (DQE) checks included item and scale distributions and a response consistency index (RCI). The online system did not allow out of range values or missing data. Scaling success was evaluated against assumptions of summated rating scales. Internal consistency reliability used Cronbach’s α. Test-retest reliability used intra-class correlations (ICC) between baseline and one-month follow-up scores among a stable disease subgroup (n = 180). Scale convergent and discriminant validity was tested and used correlations between scores from the SF-36v2, the Kansas City Cardiomyopathy Questionnaire (KCCQ-12), Patient Global Assessment of Functioning (GAF), and other surveys. Known-groups validity tests used ANOVA of scores across patient groups varying in disease severity (self-reported hematologic response status, and Patient Global Impression – Severity (PGI-S)).

RESULTS: DQE showed excellent response distribution and RCI (94.1%). Scale reliability (Cronbach’s α ≥ 0.780 across all eight domains) and test-retest reliability (ICC ≥ 0.731) were acceptable. Tests of summated rating scale assumptions were satisfactory. Scale convergent and discriminant validity showed strong correlations with conceptually related measures. Tests for known-groups validity showed that the mean scores for respondents with self-reported complete hematologic response or remission were significantly greater than scores for respondents with no response to treatment (p < 0.05 for all scores). Similarly, mean scores were also significantly associated with responses to the PGI-S (p < 0.0001 for all scores).

DISCUSSION & CONCLUSIONS: This study provided robust evidence of the psychometric properties of the SF-36v2 in a diverse sample of patients with AL amyloidosis. Planned future analyses will assess responsiveness and confirm psychometric properties of the SF-36v2 in clinic-based samples of AL amyloidosis patients.

Study supported by: Prothena Biosciences Inc
RECRUITMENT OF LIGHT CHAINS BY HOMOLOGOUS AND HETEROLOGOUS FIBRILS SHOWS DISTINCTIVE KINETIC AND CONFORMATIONAL SPECIFICITY.

Luis M. Blancas-Mejía and Marina Ramirez-Alvarado

Department of Biochemistry and Molecular Biology, 1 Department of Immunology. Mayo Clinic, 200 First St. SW, Rochester, MN 55905. USA
ramirezalvarado.marina@mayo.edu

INTRODUCTION: Light chain (AL) amyloidosis is a protein misfolding disease where immunoglobulin light chains aggregate as insoluble fibrils that accumulate in extracellular deposits. Amyloid fibril formation in vitro has been described as a nucleation-polymerization, auto-catalytic reaction where nascent fibrils catalyze formation of new fibrils, recruiting soluble protein into the fibril. In this context, it is also established that preformed fibrils or “seeds” accelerate fibril formation. In some cases, seeds of proteins with substantially different sequence are able to accelerate the reaction albeit with a lower efficiency.

MATERIAL & METHODS: In this work, we studied the recruitment and addition of monomers in the presence of seeds of five immunoglobulin light chain proteins, covering a broad range of protein stabilities and amyloidogenic properties.

RESULTS: Our data reveal that in the presence of homologous or heterologous seeds, the fibril formation reactions become less stochastic compared to de novo reactions. The kinetics of the most amyloidogenic proteins tested (AL-T05 and AL-09) do not present significant changes in the presence of seeds. The amyloidogenic protein AL-103 presented fairly consistent acceleration in presence of all seeds. In contrast, the less amyloidogenic proteins (AL-12 and κI) presented dramatic differential effects dependent of the kind of seed used. κI in general had a poor efficiency to elongate preformed fibrils.

DISCUSSION & CONCLUSIONS: Together, these results indicate that fibril formation is kinetically determined by the conformation of the amyloidogenic conformational precursor and modulated by the differential ability of each protein to either nucleate or elongate fibrils. We observe morphological and conformational properties of some seeds that do not favor elongation with some proteins, resulting in a delay in the reaction.

Fig. 1: (A) Self and cross-seeding experiments of AL-12. de novo at pH 2.0 (black), AL-09 seeds (red), AL-103 seeds (green), AL-12 seeds (blue), κI seeds (brown) and AL-T05 seeds (orange). All reactions were performed with 20 μM protein and in presence of 1% of seeds. Transmission electron microscopy (TEM) images of AL-09 at the endpoint of the reaction. (B) De novo experiment at pH 2.0 and in presence of (C) AL-09 seeds, (D) AL-103 seeds, (E) AL-12 seeds, (F) κI seeds and (G) AL-T05 seeds. Scale bar represents 200 nm.
Interim analysis of phase I study of chimeric fibril-reactive monoclonal antibody 11-1F4 in patients with AL amyloidosis

AL Langer1, J Gould2, S Miao3, MY Mapara2, J Radhakrishnan4, M Maurer5, S Raza2, JG Mears2, J Wall6, A Solomon6, Suzanne Lentzsch2

1 Department of Medicine, Columbia University, New York, United States. 2 Division of Hematology/Oncology, Columbia University, New York, United States. 3 College of Medicine, Drexel University, Philadelphia, United States. 4 Division of Nephrology, Columbia University, New York, United States. 5 Division of Cardiology, Columbia University, New York, United States. 6 Graduate School of Medicine, University of Tennessee, Knoxville, United States.

sl3440@cumc.columbia.edu

Background: The murine (Mu) monoclonal antibody (mAb) 11-1F4 prepared against human light-chain-related fibrils recognizes an amyloid-associated conformational epitope, and when administered to mice bearing human AL amyloidomas elicited a neutrophil/macrophage response that led to rapid and complete elimination of the amyloid masses with no evidence of toxicity. PET/CT imaging using I-124 labeled Mu 11-1F4 revealed uptake in the organs known to contain amyloid in the majority of patients. The NCI’s Biological Resource Branch funded the production chimeric (Ch) 11-1F4 IgG1 mAb by for a Phase I clinical trial in AL amyloidosis.

Methods: This is an open-label, dose-escalation Phase I clinical trial of Ch 11-1F4 in patients with relapsed or refractory AL amyloidosis. The trial was conducted under the auspices of an Experimental Therapeutic Investigational New Drug (IND) to define the maximum tolerated dose, and tolerability, and safety of Ch 11-1F4. Secondary objectives included pharmacokinetics, safety, and organ response. Patients were eligible if they had received prior therapy for relapsed, or refractory AL amyloidosis, or were ineligible for standard therapy, and had life expectancy of at least 3 months. Patients were excluded if they had ventricular ejection fraction less than 40%, an interventricular septal thickness greater than 25 mm, a history of sustained ventricular tachycardic or cardiac arrest, 24 hour creatinine clearance of less than 30 cc/min, alkaline phosphatase greater than three times the upper limit of normal, or total bilirubin greater than 3 mg/dL. A dose-escalation “up and down” design was used with 7 sequential doses of 0.5, 5, 10, 50, 100, 250 and 500 mg/m2. For Phase 1a, patients received a single dose of Ch 11-1F4, while in Phase 1b patients received four, once weekly doses over the course of four weeks.

Results: As of March 1st, 2016, phase 1a was completed with eight patients and phase 1b accrued 6 patients up to dose level 5 with the expectation to accrue through dose level 7, if tolerated, by May, 2016. In phase 1a, five patients had primarily cardiac, two patients primarily skin, and one primarily gastrointestinal (GI) involvement. All patients tolerated the dose received. One patient experienced a grade 3 adverse event (AE) pruritus. A skin biopsy revealed previously undiagnosed cutaneous amyloid deposits, a neutrophilic infiltrate, and histochemical evidence of Ch 11-1F4 bound to a subset of amyloid deposits, particularly in the subcutaneous fat. These findings suggest a cutaneous response. No grade 4 AEs or deaths occurred. Though the primary objective was to evaluate safety, four of eight patients of the phase 1a had evidence of organ response after only one infusion of Ch 11-1F4. At the time of presentation we will also report the interim analysis of phase 1b subjects.

Conclusions: To date, Ch 11-1F4 was well tolerated by all study subjects without any dose-limited AE’s and promising organ response after completion of phase 1a. Development of an AL-fibril-specific mAb treatment would be an invaluable adjunct in the treatment of patients with AL amyloidosis and would lead to improved medical management of this incurable and ultimately fatal disease. Clinical Trial Information: NCT02245867

**Improved survival in AL amyloidosis: a population based study on 1,492 patients diagnosed in Sweden 1990-2013**

BM Weiss¹, SH Lund², M Björkholm³, I Turesson⁴, AD Cohen¹, LM Dember¹,⁵, O Landgren⁶, SY Kristinsson²,³

¹Penn Amyloidosis Program, Division of Hematology-Oncology, Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA, USA; ²University of Iceland, Faculty of Medicine, Reykjavik, Iceland ³Karolinska Institute, Department of Hematology, Stockholm, Sweden; ⁴Skane University Hospital, Department of Hematology and Coagulation Disorders Malmö, Sweden; ¹,⁵Division of Nephrology, University of Pennsylvania, Philadelphia, PA, USA, ⁶Memorial Sloan-Kettering Cancer Center, Myeloma Service

brendan.weiss@uphs.upenn.edu

**INTRODUCTION:** AL amyloidosis (AL) is a plasma cell disorder characterized by life-threatening vital organ dysfunction resulting in nearly a third of patients dying within the first year of diagnosis. The only available therapies are anti-plasma cell chemotherapy agents, which reduce the toxic and amyloidogenic immunoglobulin light chains. We have previously shown improved survival in multiple myeloma (MM) due to novel anti-plasma cell therapies¹. Studies from specialty amyloid centers have also shown improved survival in AL, but this has never been studied in a population-based setting²,³. **MATERIALS & METHODS:** By using the nationwide Swedish Patient Registry we identified all individuals registered with AL amyloidosis (defined as more than one occurrence of the ICD-code E85.8 and E85.9) in Sweden 1990-2013. By using the Total Population Registry database we identified four matched controls for each case of amyloidosis, matched by gender and year of birth, and the controls had to be alive at the time of diagnosis for the corresponding AL-amyloidosis case. By using the Cause of Death Registry we obtained information on date of death, with follow-up through 2013. Overall survival was analyzed using Kaplan-Meier method and Cox proportional model. The cohort was divided into 5 calendar periods. **RESULTS:** We identified 1,492 AL patients; mean age at diagnosis of 65.8 years; male gender 57.8%. Compared to matched controls, AL patients in the entire cohort had a median survival of 1.73 years compared to 16.49 years for controls (p<0.001). Median overall survival for AL patients improved significantly over time and was 1.54 years for 1990-94, 0.79 years for 1995-99, 1.51 years for 2000-04, 2.23 years for 2005-09, and not reached 2010-2013 (p for trend <0.001). Survival improvements over time were seen in both those younger and older than age 65. The 1-year survival remained essentially unchanged. The 2-year survival shows a trend toward improvement. **DISCUSSION & CONCLUSIONS:** In the first population-based study of outcomes in AL, overall survival has improved from 1990-2013, likely due to the availability of highly-effective anti-plasma cell agents. Early mortality remains unchanged and unacceptably high, suggesting that early diagnosis is critical to improve outcomes in AL⁴.


**Table:** Survival in 1,492 AL-amyloidosis diagnosed in Sweden 1990-2013, by calendar period of diagnosis

<table>
<thead>
<tr>
<th>Calendar period</th>
<th>Median survival (95% CI)</th>
<th>1-yr survival (95% CI)</th>
<th>2-yr survival (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990-1994</td>
<td>1.54 (0.98-2.72)</td>
<td>0.61 (0.50-0.75)</td>
<td>0.38 (0.27-0.53)</td>
</tr>
<tr>
<td>1995-1999</td>
<td>0.79 (0.68-0.98)</td>
<td>0.42 (0.36-0.50)</td>
<td>0.30 (0.24-0.37)</td>
</tr>
<tr>
<td>2000-2004</td>
<td>1.51 (1.28-1.91)</td>
<td>0.59 (0.54-0.64)</td>
<td>0.44 (0.39-0.49)</td>
</tr>
<tr>
<td>2005-2009</td>
<td>2.23 (1.60-3.00)</td>
<td>0.60 (0.55-0.65)</td>
<td>0.52 (0.47-0.57)</td>
</tr>
<tr>
<td>2010-2013</td>
<td>Not reached</td>
<td>0.69 (0.64-0.74)</td>
<td>0.64 (0.55-0.66)</td>
</tr>
</tbody>
</table>
AL amyloidosis: real world evidence from Argentina

EM Nucifora, MA Aguirre, N Schutz, D Fantl, J Arbelbide, V Otero, P Sorroche, C Belziti, G Greloni, D Perez de Arenaza, H Garcia Rivello, DH Giunta, BR Boietti, MS Saez, ML Posadas Martinez

Department of Medicine, Instituto Universitario Hospital Italiano de Buenos Aires, Argentina. Grupo de Estudio de Amiloidosis, elsa.nucifora@hospitalitaliano.org.ar

Background: AL amyloidosis is a challenge in clinical practice due to the difficulties in diagnosis and treatment1,2. We present our experience from the Institutional Registry of Amyloidosis (RIA - NCT01347047) managing this patients.

Aims: To evaluate the characteristics and outcomes of patients with AL amyloidosis in an university tertiary hospital in Argentina.

Methods: Ambispective cohort observational study of patients with AL amyloidosis included in the RIA since January 2006 to January 2016. All data was collected following a standardized evaluation by members of the Hospital Italiano Amyloidosis Group. Data were analysed with STATA using conventional descriptive statistics.

Results: We included 165 patients during this period: AL 34.5% (57), localized 23.6% (39), unidentified 14.5% (24), senile 13.9% (23), AA 8% (13) and ATTR 5.5% (9).

Of AL amyloidosis patients 58% (33) were males, with median age of 64 years (range 35-88). Initial symptoms included heart failure 61% (35); proteinuria 65% (37); nephrotic syndrome 52% (30); renal failure 75% (43); peripheral neuropathy 26% (15) and gastrointestinal complaints 33% (19).

All patients had detectable clonal free light chains by immunofixation or serum free light chain assays. The most frequent light chain involved was Lambda 68% (39). Data regarding proBNP or BNP were available in 50 patients and only 8% (4) patients had normal results. In the laboratory workup only 44% (21/47) of patients had a measurable M protein spike.

From the 36 patients treated in our hospital: 69.4% (25) patients received dose modified CYBORD and then four received upfront cardiac transplantation; 16.6% (6) patients received IMIDs and 13.8% (5) conventional chemotherapy (prior to 2008); 26 completed full dose treatment. Autologous bone marrow transplantation after CYBORD was performed in 25% (9) patients. All of them remain in complete remission.

Eight patients older than 70 years were treated with CYBORD, 3 are in complete remission, 3 in complete remission died for cardiac failure.

Due to delay in the diagnosis 20% (11) patients died before starting proper treatment and 14% (8) that received treatment died: one in complete response and 7 in partial response. The main causes of death were 3 cardiac failure, 4 sepsis and 1 unrelated cause to amyloidosis. The median overall survival of patients treated in our hospital was 7.2 years (IC95 2.2 – 12).

Summary/Conclusion: AL Amyloidosis is underdiagnosed in Argentina. The consequent delay in diagnosis and start treatment has a huge impact in the quality of life and survival. Modified dose CYBORD is a good treatment option with and adequate response rate and safety profile considering the difficulties associated with amyloid organ damage. Free light chains response do not always correlates with organ response which usually occurs much later. Therefore it is important to consider heart involvement as an emergency. The treatment of amyloidosis AL improves the survival and quality of life.

References:


The effect of bone marrow plasma cell burden on survival in patients with AL amyloidosis undergoing high dose melphalan and autologous stem cell transplantation

C Dittus1, N Uwumugambi2, F Sun1, JM Sloan1, V Sanchorawala1

1Amyloidosis Center, Boston University School of Medicine and Boston Medical Center, Boston, MA. 2Department of Medicine, Boston University School of Medicine and Boston Medical Center, Boston, MA.

cedittus@gmail.com

INTRODUCTION: Prognosis in AL amyloidosis has been linked to several variables including: poor performance status, severe postural hypotension, New York Heart Association functional class 3 or higher, low systolic blood pressure, and higher serum free light chain concentrations. Bone marrow plasma cell (BMPC) burden has also been identified as a negative prognostic factor. Specifically, patients with a BMPC burden >10% at presentation were shown to have decreased survival (16 months) when compared to those with a BMPC burden ≤10% (46 months). In patients who received high dose melphalan and stem cell transplantation (HDM/SCT), the 5-year OS was 46% in patients with BMPC burden >10% and 73% in those with BMPC burden ≤10%. We reviewed our database to see if our experience correlates with these findings. Additionally, we assessed whether induction therapy improved survival in patients with a BMPC burden >10%.

MATERIALS AND METHODS: We reviewed 548 cases of AL amyloidosis who received HDM/SCT from 1994 through 2014. We searched for the following variables: Day 0 of SCT, date of death, BMPC burden, and induction therapy status. Kaplan Meier (KM) survival curves were calculated for overall survival in patients with BMPC ≤10%, and for those with >10%. In patients with a BMPC burden >10%, we compared KM survival curves for those who received induction therapy versus those who did not. Patients with a BMPC burden >30% were excluded from this study.

RESULTS: Of the 548 cases reviewed, 443 (81%) had a BMPC burden ≤10%, and 105 (19%) had a BMPC burden >10%. KM analysis for patients with a BMPC ≤10% revealed a median survival of 7.86 years (95% CI 6.69, 9.83), and for patients with a BMPC >10% the median survival was 6.8 years (95% CI 5.75, 11.32). There was no statistically significant difference between the two groups (HR: 1.106; CI 0.82, 1.491; p=0.51). Five-year overall survival was comparable between the two groups: 63% for those with BMPC ≤10%, and 64% for those with BMPC >10%. For patients with a BMPC burden >10% (N=105), 25 patients received induction therapy. Induction regimens included oral melphalan with prednisone (N=6), bortezomib with dexamethasone (N=16), and CyBorD (cyclophosphamide, bortezomib, dexamethasone, N=3). The use of induction therapy did not have an effect on overall survival (p=0.35) in this group with BMPC >10%.

DISCUSSION AND CONCLUSIONS: In contrast to the prior study by Kourelis et al., our study did not find a statistically significant difference in OS when stratified by BMPC burden. With a 5-year OS of 64%, our patients with a BMPC burden >10% had better survival when compared with the 46% 5-year OS in transplanted patients in that study. The lack of a statistically significant difference between groups in our study is likely because of the relatively good survival of our patients with BMPC burden >10%. The median OS of 6.8 years and 7.86 years are similar or improved compared to an earlier study published from our Center. Our study showed no benefit when patients with BMPC burden >10% received induction therapy. This supports the notion that patients with AL amyloidosis who are eligible for HDM/SCT, should not have this delayed for the administration of induction therapy.


This research was supported by the Amyloid Research Fund at Boston University.
PHASE 3 STUDY OF THE ORAL PROTEASOME INHIBITOR IXAZOMIB FOR RELAPSED/REFRACTORY AL AMYLOIDOSIS: TOURMALINE-AL1

Giampaolo Merlini,1 Angela Dispenzieri,2 Deborah Berg,3 Huyuan Yang,3 Douglas V. Faller,3 Raymond L. Comenzo4

1University Hospital San Matteo, Pavia, Italy; 2Mayo Clinic, Rochester, MN, USA; 3Millennium Pharmaceuticals, Inc., Cambridge, MA, USA, a wholly owned subsidiary of Takeda Pharmaceutical Company Limited; 4Tufts University School of Medicine, Boston, MA, USA.

gmerlini@unipv.it

INTRODUCTION: Systemic light-chain (AL) amyloidosis is a rare protein misfolding disorder caused by abnormal immunoglobulin light chains leading to organ damage. There are no approved therapies, but standard treatments utilized include chemotherapy, autologous stem cell transplantation and, more recently, the proteasome inhibitor (PI) bortezomib, and the immunomodulatory drugs lenalidomide and pomalidomide. However, AL amyloidosis remains an incurable condition and new treatment options are needed. In the phase 1 C16007 study of weekly oral ixazomib in 22 patients with relapsed/refractory AL amyloidosis (RRAL),1 the maximum tolerated dose was determined as 4 mg; overall hematologic response rate was 52% at this dose level, and a cardiac/renal response was observed in 45%/45% of patients. After a median follow-up of 37.5 months, the 9 patients who achieved ≥VGPR had a median progression-free survival of 17.0 months compared with 10.7 months in patients who achieved a lesser response (p=0.1968). These results formed the rationale for the phase 3, open-label, randomized, multicenter TOURMALINE-AL1 (C16011; NCT01659658) study and led to FDA Breakthrough Therapy Designation for ixazomib, the first investigational therapy for AL amyloidosis to receive such status.

MATERIAL & METHODS: PI-naïve patients with RRAL, who have received 1 or 2 prior therapies, have measurable major organ (heart/kidney) involvement, and ECOG performance status 0–2 are eligible. Patients are receiving oral ixazomib in combination with dexamethasone: 28-day cycles of ixazomib 4 mg on days 1, 8, and 15, and dexamethasone 20 mg on days 1, 8, 15, and 22 (dose can be increased to 40 mg after 4 weeks if tolerated). The comparator arm is physician’s choice of treatment (dexamethasone, dexamethasone plus alkylating agent, or dexamethasone plus immunomodulatory drug): 28-day cycles of dexamethasone 20 mg on days 1, 8, 15, and 22 (dose can be increased to 40 mg after 4 weeks if tolerated) alone or in combination with melphalan 0.22 mg/kg on days 1–4, cyclophosphamide 500 mg on days 1, 8, and 15, thalidomide up to 200 mg/day, or lenalidomide 15 mg/day for 21 days. The primary endpoints are overall hematologic response rate and 2-year vital organ deterioration and mortality rate. Main secondary endpoints include complete hematologic response rate, organ response rate, duration of response, progression-free survival, overall survival, time to progression, safety, quality of life, and pharmacokinetics. Importantly, the trial is the first to prospectively use the new hematologic and cardiac response criteria.2,3

RESULTS: An estimated 248 patients are planned to be enrolled; recruitment is ongoing.

DISCUSSION & CONCLUSIONS: The phase 3 TOURMALINE-AL1 study is assessing the efficacy and safety of the oral PI in patients with RRAL.

Characterization of patients with predominantly intramural coronary amyloidosis

F Kamdar, J Valent, CD Tan, ER Rodriguez, M Hanna

1Heart Failure Section, Department of Cardiovascular Medicine, Cleveland Clinic, Cleveland, USA.
2Department of Hematology and Oncology, Cleveland Clinic, Cleveland, USA, 3Department of Pathology, Cleveland Clinic, Cleveland, USA.
Kamd0001@umn.edu

INTRODUCTION: Cardiac amyloidosis occurs due to abnormal amyloid fibril deposition in the myocardium resulting in increased wall thickness. Rarely, amyloid fibrils can deposit predominantly in the coronary microvasculature with minimal interstitial deposits. We sought to characterize the clinical and echocardiographic parameters and outcomes of patients with intramural coronary amyloid deposition.

MATERIALS & METHODS: We reviewed patients with AL cardiac amyloidosis diagnosed by endomyocardial biopsy at a single institution from 2007-2015. We characterized patients with pathology demonstrating predominant intramural coronary amyloid deposition.

RESULTS: Of 265 patients with AL cardiac amyloidosis, we identified 5 (1.9%) who had cardiac amyloidosis with predominant intramural coronary deposition. All 5 patients had LVEF ≤ 40%, maximal wall thickness ≤ 1.3cm, and presented with cardiogenic shock (Table 1). ECG findings included low voltage in 4 of 5 patients. Two patients required an intra-aortic balloon pump and were bridged to a left ventricular assist device (LVAD). Three died within weeks of diagnosis.

DISCUSSION & CONCLUSIONS: Cardiac amyloidosis can uncommonly present with cardiogenic shock due to predominant intramural coronary deposition leading to global ischemia. Due to minimal interstitial deposition, these patients present with normal or mildly increased wall thickness. An ECG showing low voltage can be an important clue and the measurement of serum free light chains is an important noninvasive screen in this setting. Predominant intramural coronary deposition of amyloid should be considered in the differential diagnosis of patients with cardiogenic shock even with normal wall thickness.

Table 1: Clinical and echocardiographic parameters of patients with predominantly intracoronary deposition of AL amyloid

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>LVEF (%)</th>
<th>Maximum Wall thickness (cm)</th>
<th>LV end diastolic dimension (cm)</th>
<th>Low voltage ECG</th>
<th>Serum Light chains at diagnosis</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>M</td>
<td>30</td>
<td>1</td>
<td>4.4</td>
<td>yes</td>
<td>Kappa 1240</td>
<td>LVAD</td>
</tr>
<tr>
<td>58</td>
<td>F</td>
<td>45</td>
<td>1.3</td>
<td>4.0</td>
<td>yes</td>
<td>Lambda 228</td>
<td>LVAD</td>
</tr>
<tr>
<td>80</td>
<td>M</td>
<td>40</td>
<td>1.0</td>
<td>5.4</td>
<td>no</td>
<td>Kappa 553</td>
<td>Death</td>
</tr>
<tr>
<td>58</td>
<td>F</td>
<td>20</td>
<td>1.3</td>
<td>4.6</td>
<td>yes</td>
<td>Lambda 160</td>
<td>Death</td>
</tr>
<tr>
<td>75</td>
<td>M</td>
<td>40</td>
<td>1.3</td>
<td>4.0</td>
<td>yes</td>
<td>Lambda 1914</td>
<td>Death</td>
</tr>
</tbody>
</table>
Left Ventricular Assist Device (LVAD) for cardiogenic shock in a rare case of intracoronary light chain amyloidosis

F Kamdar¹, C Samaras², J Valent², ER Rodriguez³, CD Tan,³ N Moazami,¹ M Hanna¹

¹Heart Failure Section, Department of Cardiovascular Medicine, Cleveland Clinic, Cleveland, USA.
²Department of Hematology and Oncology, Cleveland Clinic, Cleveland, USA, ³Department of Pathology, Cleveland Clinic, USA.
Kamd0001@umn.edu

INTRODUCTION: Light chain amyloidosis (AL) is a multiorgan systemic disease due to amyloid fibril deposits derived from monoclonal immunoglobulin light chains. The disease can cause restrictive cardiomyopathy and heart failure. Rarely, the amyloid deposits can be predominantly in the cardiac microvasculature, resulting in global ischemia and cardiogenic shock. LVADs are unlikely to be considered in AL cardiac amyloidosis due to the concerns of small LV size and need for ongoing chemotherapy.

CASE: A 75 year-old man presented with exertional dyspnea. Cardiac catheterization revealed a mid LAD stenosis for which he underwent percutaneous coronary intervention. He returned 4 months later with worsening dyspnea and despite a patent stent was found to be in cardiogenic shock requiring an intraaortic balloon pump (IABP). Echocardiogram showed LVEF of 30%, normal wall thickness, LV end diastolic dimension of 4.4cm, and a restrictive filling pattern. ECG showed low voltage and poor R wave progression. Serum free light chains were obtained and demonstrated very elevated Kappa highly suggestive of AL amyloid. Endomyocardial biopsy confirmed extensive amyloid deposits in the intracoronary microvasculature with limited interstitial involvement (Figure 1). He was started on bortezomib, cyclophosphamide, and dexamethasone with a significant reduction in kappa light chains (1240 to 110 mg/L). He remained intra-aortic balloon pump dependent. Ultimately, the decision was made to pursue LVAD as destination therapy. He underwent successful implantation of a HeartWare LVAD and was able to continue chemotherapy for AL amyloidosis.

DISCUSSION & CONCLUSIONS: Predominant intramural coronary deposition of AL amyloid is rare and can present as global ischemia leading to cardiogenic shock. It is feasible for a patient with cardiogenic shock due to AL cardiac amyloidosis to undergo implantation of an LVAD and continue chemotherapy.

Fig. 1: Photomicrographs of histological sections of endomyocardial biopsies stained with H&E, thioflavin-S, amyloid kappa, and tricrome demonstrating a predominantly intramural coronary amyloid deposition.
AL Amyloidosis (ALA) as relapse of symptomatic multiple myeloma (MM): dual clinical and biological features and outcome

N.Lombion¹, M.Vignon¹, B.Arnulf¹

¹Department of Hematology and Clinical immunology, Saint Louis Hospital, Paris, France.
Marguerite.vignon@aphp.fr

AL-A is more often associated with monoclonal gammopathy of undetermined significance or smoldering MM. AL-A may also occur in the course of MM but is often asymptomatic. However, ALA related organ disease might also represent the main clinical feature of symptomatic MM at relapse.

**Patients and Method:** We retrospectively analysed a series of symptomatic MM patients who relapsed with ALA related disease as the main feature without any evidence of AL-A at MM diagnosis.

**Results:** We identified two distinct presentations. In eleven patients (group 1) AL-A occurred after a short period of time after MM diagnosis (median time 18 months), progressed rapidly resulting in a poor prognosis for some of them. In the other twelve patients (group 2), ALA occurred after a long period of time after MM diagnosis (median time 92.5 months) with a slower evolution. Patients of group 1 had higher tumor burden and poorer prognostic at diagnosis of symptomatic multiple myeloma and more often cardiac involvement at relapse as AL-A.

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=23)</th>
<th>Group 1 (n=11): Fast progressive ALA</th>
<th>Group 2 (n=12): Slow progressive ALA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age</td>
<td>59</td>
<td>68</td>
<td>55</td>
</tr>
<tr>
<td>Sex: Male/Female</td>
<td>10/13</td>
<td>6/5</td>
<td>4/8</td>
</tr>
<tr>
<td>LC isotype κ/λ</td>
<td>12/11</td>
<td>6/5</td>
<td>6/6</td>
</tr>
<tr>
<td><strong>ISS score at diagnosis:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 1</td>
<td>7/17 (41,2%)</td>
<td>4/11 (36,5%)</td>
<td>3/6 (50%)</td>
</tr>
<tr>
<td>- 2</td>
<td>4/17 (23,5%)</td>
<td>2/11 (18,2%)</td>
<td>2/6 (33,3%)</td>
</tr>
<tr>
<td>- 3</td>
<td>6/17 (35,3%)</td>
<td>5/11 (45,5%)</td>
<td>1/6 (16,7%)</td>
</tr>
<tr>
<td><strong>AL-A manifestation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac involvement</td>
<td>60%</td>
<td>63%</td>
<td>58%</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>48%</td>
<td>18%</td>
<td>75%</td>
</tr>
<tr>
<td>Neurological symptoms</td>
<td>43%</td>
<td>54%</td>
<td>33%</td>
</tr>
</tbody>
</table>

Table 1: clinical presentation at diagnosis of symptomatic myeloma and AL-A for the two groups.

The 5-years PFS and OS differed in the two groups: PFS rate was respectively of 0% for the fast progressive group versus 50% for the slow progressive group (p=0,0001). The 5-years OS rate was also significantly higher in the slow progressive group with a 90,9% rate versus 44,4% in the fast progressive group (p=0,0102).

**Conclusion:** We showed dual features of AL amyloidosis as MM relapse in term of clinical and biological characteristics and outcome. We recommend ALA investigations in front of atypical features or organ failure occurring in the course of MM.
Patterns of AL amyloid neuropathies associated with monoclonal IgM gammopathy

B Bardel¹, JP Lefaucheur², F Le Bras³, V Frenkel⁴, T Damy⁵, H Salhi¹, V Plante-Bordeneuve¹

¹ Department of Neurology, Henri Mondor Hospital, Créteil, France. ² Department of Neurophysiology, Henri Mondor Hospital, Créteil, France. ³ Department of Lymphoid Hemopathy, Henri Mondor Hospital, Créteil, France. ⁴ Department of Immunology, Henri Mondor Hospital, Créteil, France. ⁵ Department of Cardiology, Henri Mondor Hospital, Créteil, France.

INTRODUCTION: AL amyloidosis seldom occurs in IgM gammopathy and can be responsible for peripheral neuropathies, which are rarely described in the literature. The goal of this study was to assess the clinical and electrophysiological patterns of AL Amyloid neuropathies associated with IgM gammopathy in a large monocentric cohort.

MATERIAL & METHODS: We retrospectively analyzed the data of patients diagnosed with an IgM gammopathy detected by immunofixation from January 2010 to September 2015. Data were collected from the files and databases of the departments involved in the patients’ care, i.e., Neurology, Neurophysiology, Hematology, and Cardiology units at the University Hospital Henri Mondor, Paris.

RESULTS: Among the 596 individuals diagnosed with IgM gammopathy, AL amyloidosis was histologically confirmed in 9 patients (1.5%, 7 males and 2 females, mean age 66 years old [range 49 – 89]) with a Kappa (4 cases) or Lambda (5 cases) light chain. Six of them were diagnosed with Waldenström disease (mean IgM levels of 15 g/L).

Inaugural manifestations were cardiac (5 patients), neuropathic (2 patients), or renal (2 patients). Amyloid deposits were detected in salivary glands (p=5), cardiac tissue (p=2), kidney tissue (p=1), or abdominal subcutaneous fat tissue (p=1).

Of the 6 patients with available data, 4 presented symptoms of a small fiber neuropathy revealed by burning pain sensations for 2 patients, dysautonomia for 1 patient, and isolated thermoalgic hypoesthesia for 1 patient. Small fiber neuropathy was confirmed clinically and by neurophysiological investigations (laser-evoked potentials, heat detection, sympathetic skin response). In addition, 3 of them also presented sensory symptoms of large fiber neuropathy (ataxia, paresthesia, numbness) with altered nerve conduction studies. The PND score was 1 or 2 for all 4 patients. During the course of the neuropathy, severe dysautonomia occurred in 3 cases, including orthostatic hypotension and gastrointestinal manifestations. Overall, neuropathic symptoms occurred on average 9 months (range 1 – 19) before biological or histological proof.

During the follow-up, other organs affected by amyloidosis were the heart (7 patients), kidneys (3 patients), muscles (1 patient), the pleura (1 patient) and the liver (1 patient). The median survival time after being diagnosed with amyloidosis was 14 months. Three patients died from complications of heart and kidney failure, while 3 patients were still alive at the end of the study.

CONCLUSIONS: Our study shows the spectrum of neuropathies detected in the course of AL amyloidosis associated with IgM gammopathy. They manifest as small fiber neuropathy with painful sensations and autonomic symptoms, often accompanied by large fiber neuropathy. While cardiac and renal manifestations of amyloidosis are generally considered as more significant, these sensory neuropathies appear to be frequent and occur several months before the amyloidosis is proven.

Symptoms of small fiber neuropathy occurring in an IgM gammopathy should systematically lead to look for AL amyloidosis to provide earlier care.
NEOD001 SPECIFICALLY BINDS AGGREGATED LIGHT CHAIN INFILTRATES IN MULTIPLE ORGANS OF PATIENTS WITH AL AMYLOIDOSIS AND PROMOTES PHAGOCYTIC CLEARANCE OF LIGHT CHAIN AGGREGATES IN VITRO

W Zago, M Renz, R Torres, SJ Tam, PJ Dolan, JR Tapia, L Li, RM Barbour, JR Salmans, PJ Shughrue, T Nijjar, D Schenk, GG Kinney

Prothena Biosciences Inc, South San Francisco, California, USA.
wagner.zago@prothena.com

INTRODUCTION: Amyloid light chain (AL) amyloidosis is the most common form of systemic amyloidosis and is characterized by the accumulation of aggregated, misfolded immunoglobulin light chains in a variety of organs, resulting in serious organ damage and dysfunction. To date, no therapies have been approved to treat patients with AL amyloidosis, and current approaches do not directly target the underlying cause of organ dysfunction. NEOD001 is a conformation-specific antibody currently in phase 2b and 3 clinical trials that specifically targets immunoglobulin light chain aggregates and may promote the clearance of AL deposits by phagocytosis. In the present study, we evaluated the binding characteristics of NEOD001 to aggregates deposited in various AL organs using immunolabeling and binding assays. We also assessed the ability of the antibody to induce AL amyloid clearance by phagocytosis in vitro.

MATERIAL & METHODS: The murine form of NEOD001, the 2A4 antibody, was used in these studies to avoid the nonspecific detection of human immunoglobulin G in human tissue. Immunohistochemical and biochemical techniques were used to characterize 2A4 immunoreactivity to AL aggregates. A total of 21 organ tissue samples derived from the heart, kidney, liver, and spleen of 10 AL patients were examined. Fluorescent and chromogenic immunohistochemistry (IHC) were performed on both fresh frozen and aldehyde-fixed tissue. Thioflavin T (ThioT) and Congo red labeling were used in parallel with IHC to identify amyloid. To assess the binding of 2A4 to extracts from patients with AL amyloidosis and recombinant light chain, we used a newly developed immunoassay specific to light chain aggregates and other biochemical readouts. Phagocytosis was assessed in vitro using a macrophage cell line cultured in the presence of amyloid deposits isolated from the heart of a patient with AL amyloidosis. All experiments included control isotype-matched antibodies.

RESULTS: 2A4 showed specific binding to both soluble and insoluble light chain aggregates isolated from the tissue of patients with AL amyloidosis but not to normally folded light chain. 2A4 specifically labeled all 21 fresh frozen tissue samples, which were derived from 10 patients with AL amyloidosis and represented both κ and λ light chain amyloidosis subtypes. No specific staining was observed in tissue samples from healthy subjects or patients with ATTR amyloidosis. Immunostaining of deposits with 2A4 was almost completely attenuated in formalin-fixed samples, even after brief fixation (1 minute). 2A4 induced the rapid engagement of macrophages and stimulated the phagocytic clearance of light chain deposits.

DISCUSSION & CONCLUSIONS: This study demonstrated that 2A4, from which NEOD001 was derived, specifically bound to amyloid light chain and soluble light chain aggregates in various organs of patients with AL amyloidosis and, in vitro, promoted the clearance of light chain aggregate particles by macrophage phagocytosis. These results demonstrate that NEOD001 directly targets the AL protein, which is known to be the underlying cause of organ dysfunction in AL amyloidosis.
A MULTIFACTORIAL DISEASE REQUIRES A MULTIDISCIPLINARY APPROACH: AN INVESTIGATION INTO IMMUNOGLOBULIN LIGHT CHAIN AMYLOIDOSIS USING A COMBINATION OF COMPUTATIONAL, CELL AND BIOCHEMICAL TECHNIQUES.

Kieran Hand, Hannah A. Davies, Daniel J. Rigden, Jillian Madine

Department of Biochemistry, Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom.

INTRODUCTION: Light chain (AL) amyloidosis involves systemic extracellular deposition of protein, causing tissue disruption and irreversible damage to virtually all organs. What remains part of an ongoing investigation is whether the initial site of fibrillation occurs intracellularly or extracellularly, and alike all proteinopathies, the atomistic details of fibril assembly and the mechanisms of cytotoxicity remain unresolved to date. In a disease where the precursor protein has over 3000 possible different amino acid combinations, exhibits multiple protein states, and manifests itself in virtually all tissues, we take a multidisciplinary approach by combining the use of computational, cell physiology and biochemical based techniques to further characterise 3 homologous Immunoglobulin light chain variable domains of the Kappa isotype.

MATERIAL & METHODS: With the use of the macromolecular modelling suite Rosetta, we computationally generate an Immunoglobulin light chain based on crystallographic models and; 1) perform in silico mutagenesis to systematically identify mutations which dramatically contribute to the thermodynamic stability of each light chain with the use of FoldX, 2) study the inter-residue relationships and their ability to dictate a canonical/non-canonical dimer interface, 3) screen for common features that may aid in the design of dimer stabilising compounds, 4) investigate the role of post-translational modifications on structural stability and 5) identify aggregation hotspots using Aggrescan3D.

RESULTS: Computational studies have allowed us to acquire structural data that has since been elusive, identify aggregation hotspots and residues that contribute to the stability of proteins both in a monomeric and dimeric form.

DISCUSSION & CONCLUSIONS: Recombinant protein expression methods refined in our laboratory means that computational findings can be further validated with cell and biochemical work. We are also investigating the ability of soluble light-chain species to internalise and further studying:1) the mechanisms of internalisation, 2) subcellular compartmentalization, which will form the motive for a pharmacological inhibition assay, 3) changes in cellular chemistry that occur in response to the uptake of exogenous protein and 4) functional perturbations in cardiac cell models. Taken together, we hope that a multi-technique based approach will allow us to identify novel cellular and common structural therapeutic targets which can aid in the treatment of light chain amyloidosis.


Fig. 1: Construction of canonical (A) and non-canonical (B) dimer states of Ig proteins. Generated homology structures based on known crystal structures were subject to energy minimisation, validated, and modelled on structures that exhibit an altered and germ-line preserved canonical dimer interface.
INTRODUCTION: Cardiac dysfunction dominates prognosis in AL amyloidosis and cardiac biomarkers (troponin and NTproBNP) can identify patients at high risk (stage 3 per Mayo stage). Low blood pressure (BP) has been associated with poor prognosis, especially in patients with Mayo stage-3 disease (Wechalekar et al Blood 2013). However, BP is not constant or fixed and varies within 24h, during day and night or during exercise. Cardiac output and autonomic nervous system (ANS) are major regulators of BP and both are affected in AL amyloidosis. We prospectively evaluated the prognostic role of BP by using standard (office) and 24-hour BP measurements. Furthermore, we evaluated the importance of the deregulation of ANS in AL amyloidosis by assessing baroreceptor reflex sensitivity (BRS).

MATERIAL & METHODS: Newly diagnosed AL amyloidosis patients were prospectively evaluated, before initiation of therapy. All patients underwent standard office BP measurements (three BP measurements taken at a 1-min intervals, at sitting position, averaged to obtain a single systolic and diastolic office BP value), 24h ambulatory BP monitoring and a simultaneously electrocardiographic and non-invasive BP monitoring (Finometer), under standardized conditions for 15 min. Ambulatory BP monitoring was performed on a usual working day. BP recordings were obtained automatically at 15-min intervals throughout the 24h period. BRS was expressed as the alpha-index (a-index), which was estimated by means of power spectral analysis.

RESULTS: So far 68 consecutive patients (median age 65, range 40-84 years, 50% males) have been evaluated. Cardiac involvement was present in 65%, kidney in 70% and peripheral or ANS in 23%. Per Mayo stage 10%, 60% and 30% were stage-1, -2 & -3 while 14% had NTproBNP≥8500 ng/L. Median office systolic BP (SBP) was 118 mmHg and median diastolic BP (DBP) was 72 mmHg. SBP was lower in Mayo stage-2 vs -1 and stage-3 vs either stage-2 or stage-1 patients (p=0.026); there was no difference in the DBP. The median of mean 24h ambulatory SBP was 112.5 mmHg and of DBP was 69.5 mmHg. Advanced Mayo stage was associated with lower mean 24h SBP (p=0.048). None of the patients with Mayo stage 1, 13% of those with stage-2 and 33% of those with stage-3 had office SBP<100 mmHg. No patient with stage-1, 23% with stage-2 and 42% with stage-3 had mean ambulatory SBP<100 mmHg. Either office SBP<100 mmHg (6 months vs not reached, p<0.001) or mean 24h SBP<90 mmHg (2 months vs not reached, p<0.001) were associated with poor survival and early death. BP fluctuations within 24h were higher in patients with less severe or no heart involvement, while more often patients with stage-3 vs stage-1 or -2 patients had higher nighttime vs daytime SBP. Median a-index was 2.85 (range 0.4-5.85): it was lower in patients with advanced cardiac involvement and in patients who had nerve involvement (median 1.6 vs 3.3, p=0.016), reflecting both cardiac and nerve dysfunction, and was associated with early death.

DISCUSSION & CONCLUSIONS: BP measurement (either office or 24 hour) provides prognostic information in patients with AL amyloidosis; 24h ambulatory measurements can provide additional data and also insights into pathophysiologic mechanisms of BP deregulation. Impaired BRS is associated with advanced cardiac and nerve involvement and risk of early death. Further analysis and additional data will be presented in the meeting.
PC25

Hematologic and renal impairment of monoclonal immunoglobulin deposition disease after treatment with bortezomib-based regimens

E Kastritis¹, DC Ziogas¹, M Gavriatopoulou¹, E Terpos¹, M Roussou¹, M Migkou¹, E Eleutherakis-Papaiovou¹, D Fotiou¹, I Panagiotidis¹, E Kafantari¹, E Psimenou¹, I Boletis², DV Vlahakos³, H Gakiopoulou⁴, CMatsouka⁵, MA Dimopoulos⁵

¹Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine, ²Department of Nephrology, “Laikon” Hospital Athens Greece, ³Renal Unit, “Attikon” General University Hospital, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece, ⁴1st Department of Pathology, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece;

ekastritis@gmail.com

INTRODUCTION: Monoclonal Immunoglobulin deposition disease (MIDD) is characterized by the deposition of monoclonal immunoglobulin chains in the kidney causing renal function deterioration. No optimal treatment has been established and recommendations supporting bortezomib-based regimens as primary choice are based on small series of patients. In addition, response criteria for the evaluation of treatment outcomes in patients with MIDD have not been formally published, although recent publications supported the implementation of hematologic response criteria based on FLCs.

MATERIAL & METHODS: We analyzed the renal and hematologic outcomes of 18 consecutive patients with biopsy proven renal MIDD who were treated homogeneously with bortezomib-based treatments, either bortezomib with dexamethasone (VD) or VD with cyclophosphamide (VCD).

RESULTS: interstitial fibrosis(78%), mesangial expansion/proliferation(72%), tubular atrophy (67%), nodular mesangial sclerosis(56%) were the most common pathologic findings. None of the patients had cast nephropathy or symptomatic multiple myeloma by other CRAB criteria. Median age was 66 years; hypertension(89%), proteinuria (median 3.5 gr/24h) and severe renal dysfunction (eGFR<30ml/min in 50%) were presenting features. Five (28%) patients had negative serum and urine immunofixation and 14 (78%) had measurable levels of FLCs (dFLC>50mg/l and abnormal ratio). Median bone marrow infiltration was 15%. Twelve patients (67%) received (VD) and 6 (33%) VCD; 8 patients (44%) received bortezomib IV and 10 (56%) SC. Three patients received high dose melphalan as consolidation after bortezomib-based induction. Overall, 11/14 (79%) evaluable patients achieved a hematologic response [5(36%) CR, 1(7%) VGPR, 5(36%) PR] after a median of 3 cycles of treatment. Six of the 9 (66%) patients with eGFR<30 ml/min increased eGFR to ≥30 ml/min (MRenal) and 10/16 (63%) with baseline proteinuria ≥1gr/24h reduced proteinuria by ≥50%. At the time of diagnosis (N=2) or during follow-up (N=4), 6 (33%) patients progressed to ESRD. No patient who achieved a hematologic VGPR or CR developed ESRD, but 4/8 patients who achieved a PR or less progressed to ESRD. Baseline eGFR<30ml/min was associated with increased risk for ESRD (OR=8.75, p=0.086); no other clinical or histologic factors were associated with increased risk for ESRD. During follow-up (median 39 months), 3(17%) patients developed hematologic relapse/progression. Peripheral neuropathy occurred in 67% (grade 2 in 7 and grade 3 in 2 patients); no patient discontinued bortezomib due to neuropathy but in 9/18 a dose reduction was required. Neuropathy grade ≥2 occurred more often in patients who received bortezomib IV than SC (87.5% vs 20%, p=0.041).

DISCUSSION & CONCLUSIONS: bortezomib-based treatment is an active and safe therapy for patients with MIDD but at least a hematologic VGPR is required in order to delay progression to ESRD and improve renal function and proteinuria.
Bortezomib before, in and after autologous stem cell transplantation in patients with newly diagnosed AL amyloidosis

Xianghua Huang¹, Qingwen Wang¹, Wencui Chen¹, Dehua Gong¹, Caihong Zeng¹, Zhihong Liu¹

¹ National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine

liuzhihong@nju.edu.cn

INTRODUCTION: In previous study, we have demonstrated that the outcome of treating AL amyloidosis with bortezomib with dexamethasone (BD) induction followed by autologous hematopoietic stem cell transplantation (ASCT) was superior to the outcome of the ASCT treatment alone. To further improve the hematologic response rate, we conducted a prospective trial of bortezomib before, in and after ASCT in patients with newly diagnosed AL amyloidosis.

MATERIAL & METHODS: Newly diagnosed AL amyloidosis patients who met the criteria of ASCT could be included in this trial. Treatment schedule consisted of two cycles of BD induction therapy (bortezomib 1.3mg/m² and dexamethasone 40 mg/d on days 1, 4, 8 and 11 followed by 10 days rest), ASCT treatment (the conditioning regimen consisted of melphalan and bortezomib, the dose of bortezomib was 1mg/m² in day -6, -3, +1, +4), and four additional 21-day cycles of bortezomib treatment (with a dose of 1.6mg/m² on day 1 and 8 of the cycle) will be conducted as consolidation therapy after ASCT. The objectives were hematologic response, tolerability and survival.

RESULTS: From March 2011 to September 2014, 18 patients were enrolled in the study. 9 patients had cardiac involvement. The overall hematologic response rate was 94.4% (17/18), including 13 patients (72.2%) with complete response, 4 patients (22.2%) with very good partial response. The organ response rate was 72.2%. The organ response was reached in 13 patients of the 18 patients with renal involvement and 7 of the 9 patients with cardiac involvement. Peripheral neuropathy and infection were the common adverse events during the treatment, and 4 patients have been discontinued bortezomib for neuropathy. No death occurred in this study. After a median follow up of 24 months, the overall survival was 100%, and the estimated progression free survival was 91% at 48 months.

DISCUSSION & CONCLUSIONS: In conclusion, our preliminary data suggest that incorporating bortezomib into induction, conditioning and consolidation with ASCT yielded a high rate of hematologic response with tolerable toxicity. (ClinicalTrial.gov Identifier: NCT01273844)
Importance of renal stage, treatment and hematologic response in the risk of end stage renal disease in patients with AL amyloidosis

M Gavriatopoulou, M Roussou, E Terpos, D Fotiou, DC Ziogas, E Eleutherakis-Papaiovou, T Apostolou, I Boletis, C Pamboukas, E Papadopoulou, E Psimenou, MA Dimopoulos, E Kastritis

1Department of Clinical Therapeutics, National and Kapodistrian University of Athens, School of Medicine; 2Department of Nephrology, Evangelismos General Hospital, 3Department of Nephrology and Transplantation, National and Kapodistrian University of Athens, School of Medicine, Athens, Greece; ekastritis@gmail.com

INTRODUCTION: Kidney is commonly involved in AL amyloidosis and many patients develop end stage renal disease (ESRD) requiring dialysis. A staging system based on the presence of reduced eGFR (<50 ml/min) and/or of significant proteinuria (>5 gr/d) at diagnosis was able to identify patients with low, intermediate or high probability of progression to dialysis. Achievement of at least hemVGPR was associated with better renal outcomes but the optimal therapy for patients presenting with renal involvement has not been defined. We validated the above prognostic system in an independent cohort and we assessed the role of different primary therapies in the risk of ESRD, using competing risk models for cardiovascular death due to cardiac amyloidosis.

MATERIAL & METHODS: We analyzed the renal and survival outcomes of 128 consecutive patients with newly diagnosed AL and kidney involvement, which were treated and followed in the Department of Clinical Therapeutics. Death before dialysis (due to cardiac amyloidosis mostly) was treated as competing event, since many patients die of complications of cardiac amyloidosis and do not develop ESRD.

RESULTS: Median age was 65 years (range 40-84), 58% had cardiac involvement, 25%, 50% and 25% were Mayo stage-1, -2, & -3. Median eGFR was 61 ml/min; 41% had eGFR<50 ml/min, 24% had eGFR<30 ml/min and 59% had proteinuria ≥5 gr/d. Eight (6%) patients required dialysis at the time of diagnosis and were excluded from further analysis. Primary therapy was bortezomib-based in 54%, lenalidomide-based in 24%, MDex in 14%, VAD in 4% and MP in 3%. After a median follow up of 4.5 years, 31% of the patients developed ESRD requiring dialysis; the 1-, 2- and 3-year dialysis rate was 18%, 20% & 28% respectively. By renal staging 23%, 57% & 20% were at low, intermediate and high risk and the 2-year dialysis rate was 4%, 18% & 36% for low, intermediate and high risk groups. After adjustment for renal stage bortezomib-based regimens were associated with lower probability of progression to ESRD than lenalidomide-based therapy (HR:0.5, p=0.045) but there was no difference between bortezomib-based regimens and MDex (p=0.51). After adjustment for renal staging the difference between different clonal response categories (CR vs VGPR vs PR) was not statistically significant. Reduction of proteinuria >30% at 3 months after initiation of therapy was associated with improved renal outcomes (HR: 0.37, p=0.047).

DISCUSSION & CONCLUSIONS: In a competing risk analysis the degree of deterioration of renal function assessed by the eGFR and the amount of proteinuria remain the most important factors predicting the risk of ESRD requiring dialysis. Our data indicate that despite effective therapy, early diagnosis is the most crucial factor and that treatment should be initiated before severe renal damage occurs, in order to reduce the risk of ESRD.
INTRODUCTION: Vascular function may be affected in patients with AL amyloidosis and vascular dysfunction may contribute to complications of AL amyloidosis, including early mortality. The aim of our study was to prospectively examine non-invasive markers of vascular damage and their clinical and prognostic implications in patients with AL amyloidosis.

MATERIAL & METHODS: We prospectively studied 122 newly diagnosed, previously untreated patients with systemic light chain (AL) amyloidosis. At baseline they were compared with control subjects matched 1:1 for traditional factors that have been correlated with vascular function including age, gender and GFR stage. In addition, 66 patients were also matched strictly 1:1 for traditional risk factors of cardiovascular disease and stage of renal dysfunction. In all patients, flow-mediated dilatation (FMD), carotid-femoral pulse wave velocity (PWV), reflected waves (AI), intima-media thickness (IMT) and the presence of plaques in the carotid and common femoral arteries were measured, before administration of any kind of therapy. The primary end-point was all-cause mortality.

RESULTS: FMD was significantly higher (p<0.05) in the AL group compared to the control group after adjustment for blood pressure (BP). Peripheral and aortic blood pressure (p=0.022 to <0.001), PWV (p=0.051), and AI (p<0.001) were significantly lower in the AL patients both in the strictly and not strictly matched populations. Median follow-up is 41 months and 38% of the patients have died. Increased FMD correlated with early mortality independently of age, gender, Mayo stage and BP (p=0.028) and improved reclassification of AL patients at risk for death (continuous NRI 55.6%, p=0.007) over a model including cardiobiomarker-based stage (Mayo stage) and systolic blood pressure. IMT in the internal carotid artery was the only atherosclerosis marker found to be significantly higher (p=0.036) in the AL group but did not correlate with adverse outcome.

DISCUSSION & CONCLUSIONS: In this prospective study which included large number of consecutive patients with AL amyloidosis we found that vascular over-reactivity, expressed as increased FMD, is associated with early mortality. More importantly, FMD correctly reclassifies risk over established risk factors in AL amyloidosis. These data indicate a mechanism of vascular dysfunction in AL and suggest that FMD may serve as a novel marker for risk stratification in AL patients.
Plasma cell clones in patients with systemic light chain amyloidosis express CS-1 but not BCMA

M. Rosenzweig,1,2 X, Wang,1 R, Urak1, M, Walter, L, Lim1, N, Nathwani1,2, M, Htut1,2, C, Karanes1,2, G, Somlo1,2, A, Krishnan1,2, S, Forman1,2

Department of Hematology and Hematopoietic Cell Transplantation, City of Hope National Cancer Center, Duarte, California, U.S.A. 2Judy and Bernard Briskin Myeloma Center.
mrosenzweig@coh.org

INTRODUCTION: The goal of therapy for light chain amyloidosis (AL) is eradication of the abnormal plasma cells through both conventional chemotherapy and autologous stem cell transplantation. Immunotherapy is an appealing approach to explore in AL because of the low burden of disease, but the optimal target remains elusive. CS1 is a cell surface glycoprotein of the signaling lymphocyte activation molecule (SLAM) receptor family that is highly and selectively expressed on normal plasma cells and multiple myeloma (MM) cells, with lower expression on NK cells and little or no expression on other normal tissue. CS1 expression on the plasma cells of patients with AL amyloidosis has been previously reported [1]. We set out to confirm expression of CS1 as an appropriate target for CAR T cells as well as further characterize the clonal cell in AL.

MATERIALS & METHODS: We performed a prospective study evaluating bone marrow specimens of patients with plasma cell diseases referred to our center (9/9/2014- present). Patients had full clinical evaluations for characterization of the hematologic clone as well as organ involvement. Using multi-color flow cytometry analysis, we set out to differentiate between malignant and normal plasma cells by analysis of aberrant ratios of intracellular kappa/lambda chains.

RESULTS: Six patients with AL amyloidosis have been enrolled on this study to date. Patient characteristics and flow cytometry results are shown in Table 1. All six demonstrated CS1 expression on the clonal plasma cells, and none expressed B cell maturation antigen (BCMA).

DISCUSSION & CONCLUSIONS: Although the number of patients is small, this study confirms expression of CS1 on the clonal plasma cells of patients with AL. It is interesting to note the lack of expression of BCMA on the plasma cells in this group of patients since BCMA is thought to be ubiquitously expressed on the plasma cells of patients with multiple myeloma [2]. Our findings suggest the clonal plasma cell seen in patients with AL is unique with respect to that of patients with multiple myeloma. In addition, our findings support further investigation of CS1-directed CAR T cell therapy for patients with AL.

Table 1: Patient characteristics and flow cytometry results

<table>
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<th>Characteristic</th>
<th>Value</th>
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<tr>
<td>Median age in years median (range)</td>
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<tr>
<td>Gender: M/F</td>
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<tr>
<td>Type of light chain: kappa/lambda</td>
<td>3/3</td>
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<tr>
<td>Median dFLC mg/dL (range)</td>
<td>22.7 (2.6-68.5)</td>
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<tr>
<td>Median % plasma cells by IHC (range)</td>
<td>12.7 (2-20)</td>
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<tr>
<td>Number with amyloidgenic clone detected by flow</td>
<td>6</td>
</tr>
<tr>
<td>Flow analysis: CS1/BCMA</td>
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<tr>
<td>FISH results: Gain of 1q21, t(4;14), hyperdiploid</td>
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REFERENCES:


Bortezomib-dexamethasone versus high-dose melphalan for Japanese patients with systemic light chain (AL) amyloidosis: A retrospective single-center study

N Katoh, Y Sekijima, M Matsuda, and S-I Ikeda

Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan
nagaaki@shinshu-u.ac.jp

INTRODUCTION

Both bortezomib-dexamethasone (Vd) and high-dose melphalan with stem cell transplantation (HDM) are reported to provide good treatment outcomes in patients with systemic AL amyloidosis. However, these two treatments have not been well compared. The aim of this study is to compare efficacy and safety of these two treatments for Japanese AL amyloidosis patients at our center.

MATERIAL & METHODS

We retrospectively investigated medical records of AL amyloidosis patients who were treated at our institution during the period of Sep. 2001 to Feb. 2016. Clinical backgrounds and treatment outcomes (efficacy and safety) of those treated by Vd or HDM were comparatively analyzed. In Vd regimen, 1.0 or 1.3 mg/m$^2$ bortezomib was subcutaneously given on day 1, 4, 8, and 11 with 40mg dexamethasone on day 1-4 every 21 days. In HDM regimen, total 140 mg/m$^2$ melphalan was given followed by autologous stem cell support with or without vincristine, doxorubicin and dexamethasone (VAD) induction therapy.

RESULTS

Twenty patients treated by Vd and thirty patients treated by HDM were found. Between two groups, there were no significant differences in clinical backgrounds including dFLC, NTproBNP, TnT, visceral organ involvements, Mayo 2012 stage, NYHA class, and performance status, except for the age, previous history of other chemotherapy, and transplant eligibility. In Vd group, the mean age was higher (63.2 vs 55.8, $P = 0.0033$), the amount of patients who had already underwent other chemotherapy was higher (35.0% vs 0.0%, $P = 0.0008$), and the number of patients who fulfilled transplant eligibility criteria was lower (65.0% vs 96.7%, $P = 0.0046$). Among those treated by Vd, treatment related mortality was lower (5.0% vs 10.0%, $P = 0.6411$), hematological response rate (partial response or better) was higher (90.0% vs 73.3%, $P = 0.2789$), and complete response rate was higher (55% vs 50%, $P = 0.7288$), although these differences were not statistically significant. Survival curves of two groups (Fig. 1) were both good (they didn’t reach 50%) and there was no significant difference ($P = 0.7176$).

DISCUSSION & CONCLUSIONS

Vd treatment was as effective and safe as HDM in this study. Notably, Vd achieved this outcome among patients with poor clinical backgrounds as compared with HDM (i.e. higher age, refractory nature to other chemotherapy, and poor transplant eligibility).

Fig. 1: Kaplan-Meier’s survival curves of the 2 treatment groups. There was no significant difference ($P = 0.7176$)
ASSOCIATION OF MULTIPLE MYELOMA WITH LIGHT CHAIN AMYLOIDOSIS - VISCERAL DAMAGES, TREATMENT AND IMPACT ON SURVIVAL: A SINGLE CENTER EXPERIENCE

Sorina Badelita, Camelia Dobrea, Andreea Jercan, Monica Popescu, Daniel Coriu
Center of Hematology and Bone Marrow Transplantation, Fundeni Clinical Institute,
University of Medicine and Pharmacy “Carol Davila”, Bucharest

Light-chain amyloidosis (AL) is a disease characterized by tissue accumulation of free light chains that form amyloid fibrils. This disease can occur independently (primary amyloidosis) or in the context of another monoclonal gammopathy, such as multiple myeloma (MM), Waldenstrom’s disease or non-Hodgkin’s lymphoma with secretion of monoclonal protein.

This retrospective study shows the impact of the association between MM and AL regarding the clinical evolution, the type of visceral amyloid involvement, the treatment response and the survival impact.

Material and Methods: a total of 266 patients with MM admitted to our clinic between 2005 and 2014; specific diagnosis and monitoring tests for multiple myeloma. Specific tests for amyloidosis: Congo Red Staining with polarized light analysis of samples from abdominal fat biopsy and/or tissue biopsy; immunohistochemistry; electron microscopy; free light chain assay; 24-hour proteinuria; EMG; Echocardiography; EKG; Fibro-Scan; cholestasis tests.

Results and discussion: AL was identified in 44 patients (16.54%) of the total of 266 patients with multiple myeloma. In this group of 44 patients with AL and MM: 66% had onset signs related to multiple myeloma, and 34% patients had onset signs related to amyloidosis (fatigue, weight loss, edema, peripheral neuropathy). 28 patients (63.63%) had at least 2 amyloidosis-involved organs. 50% of patients had renal impairment manifested by chronic kidney disease and/or nephrotic syndrome.

The treatment of patients with MM and AL was adjusted for each patient according to the respective type of visceral amyloidosis related lesion: melphalan plus dexamethasone (19.5% pts.); cyclophosphamide, bortezomib and dexamethasone (61% pts.). Autologous bone marrow transplant was performed in 34.1% of patients; all patients with cardiac amyloid involvement were excluded.

We analyzed the survival of patients with MM associated with AL comparatively to that of patients diagnosed only with MM. Mortality in MM and AL patients was 61.6% (22 of the patients died) and the median survival period was reduced from 77 months for patients diagnosed only with MM, to 44 months for patients with MM and AL. The severity of the prognosis is based on the number of involved organs: in patients with more than 3 organ damages, the median survival was reduced 4 times, to only 17 months compared to the control group (patients diagnosed only with MM), and the mortality reached almost 70% of the patients. For patients with MM and AL with cardiac involvement, survival dropped dramatically to only 10 months, and 85% of them died.

Conclusions: The association of AL amyloidosis in patients with multiple myeloma is a complication seen in 16% of our MM patients, being highly underdiagnosed in the daily medical practice, with a major impact on the evolution and survival of multiple myeloma patients.

This work was supported by the grant CEEX 74/2006 from the Romanian Ministry of Research and Technology.
Pre-transplantation novel agent induction predicts progression-free survival for patients with immunoglobulin light-chain amyloidosis undergoing high-dose therapy and autologous stem-cell transplantation

AJ Cowan1,2, PA Stevenson2, S Tuazon1,2, PS Becker2,3, DJ Green1,2, LA Holmberg1,2, DG Coffey1,2, AK Gopal1,2, EN Libby1,2

1Division of Medical Oncology, University of Washington, Seattle, WA, USA. 2Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA. 3Division of Hematology, Department of Medicine, University of Washington, Seattle, WA, USA

acowan@u.washington.edu

INTRODUCTION: High-dose melphalan and autologous stem cell transplant (HDM/SCT) is an effective treatment modality for immunoglobulin light-chain (AL) amyloidosis, however, its application remains restricted to patients with good performance status and limited organ involvement. In recent years, the paradigm for AL amyloidosis has changed with the introduction of novel agents such as immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs). We hypothesized that use of novel agent induction regimens could improve outcomes for patients with AL amyloidosis undergoing HDM/SCT.

MATERIAL & METHODS: Sequential patients with confirmed AL amyloidosis, age ≥ 18 years who underwent HDM/SCT between 2001 and 2014 at the Fred Hutchinson Cancer Research Center and University of Washington Medical Center were eligible. Any regimen administered prior to HDM/SCT including an IMiD or a PI was considered a novel induction regimen. Use of induction regimen was evaluated in a Cox proportional hazard model for association with progression-free (PFS) and overall survival (OS).

RESULTS: Forty-five patients with AL amyloidosis underwent HDM/SCT. The median age was 57.2 years (range, 39 – 74.4), 15 (33.3%) were women. The median number of organs involved was 2 (range 1 – 5), with 20 patients having only 1 (44.4%), 10 patients having 2 (22.2%), and 15 patients (33.3%) having ≥ 3 organs involved. Novel agent induction regimens were used prior to HDM/SCT in 21 patients (46.7%); these comprised PI in 12 (26.7%), IMiD and PI in 5 (23.8%), and IMiD alone in 4 (19%). Use of a novel agent induction regimen was associated with improved PFS, but not OS (Figure 1). The 3 year PFS for patients who received a novel agent induction was 79%, while for those who did not was 53% (Hazard ratio [HR] = 0.317, p = 0.048). The 3 year OS for patients who received novel agent induction regimen was 95%, while for those who did not was 71% (HR = 0.454, p = 0.247).

DISCUSSION & CONCLUSIONS: Our data suggest that use of a novel agent induction regimen including an IMiD or PI prior to HDM/SCT for patients with AL amyloidosis was associated with superior PFS, with a trend towards improved OS. Although these results are limited by sample size and lack of randomization, these results support further investigation of novel agent induction regimens in the context of a randomized clinical trial.

Figure 1. Pretransplant novel agent induction predicts PFS in patients with AL amyloidosis undergoing HDM/SCT. Kaplan-Meier plots for progression-free survival and overall survival.
Frequency of amyloid subtyping among patients with immunoglobulin light-chain amyloidosis referred for high-dose chemotherapy and autologous stem cell transplant

AJ Cowan1,2, DG Coffey1,2, PS Becker2,3, DJ Green1,2, LA Holmberg1,2, AK Gopal1,2, EN Libby1,2

1Division of Medical Oncology, University of Washington, Seattle, WA, USA. 2Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA. 3Division of Hematology, Department of Medicine, University of Washington, Seattle, WA, USA

acowan@u.washington.edu

INTRODUCTION: The amyloidoses comprise a heterogeneous group of diseases characterized by misfolding of amyloidogenic proteins and subsequent deposition as amyloid fibrils. To date, over 30 proteins are known to be amyloidogenic (1). Immunoglobulin light chain (AL) amyloidosis, a plasma cell dyscrasia, is the most common subtype. In eligible patients, high dose melphalan and autologous stem cell transplant (HDM/SCT) is preferred; novel agents such as immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs) are also effective. Accurate subtyping of amyloidosis is essential to appropriate treatment, as misdiagnosis occurs in up to 10% of patients and may lead to inappropriate administration of chemotherapy (2, 3). We sought to determine the patterns of amyloid subtyping among patients with a diagnosis of AL amyloidosis referred to a tertiary referral center for HDM/SCT.

MATERIAL & METHODS: Sequential patients with confirmed amyloidosis, age ≥ 18 years who underwent HDM/SCT between 2001 and 2014 at the Fred Hutchinson Cancer Research Center and University of Washington Medical Center were eligible. Presence of a Congo red-positive biopsy for each patient referred for transplant was confirmed and the pathology reports and medical records were reviewed to determine if subtyping was performed, and which modality was used.

RESULTS: Fifty-one patients with AL amyloidosis were referred for transplant; of these, 45 proceeded with HDM/SCT. The organ systems most commonly involved were renal in 34/51, and gastrointestinal in 5/51. Of the biopsies, subtyping was performed in 35 (68.6%), and no subtyping was performed in 16 patients (31.3%). Immunofluorescence was the most common modality used for subtyping in 33 biopsies (94.2%) and laser capture/mass spectrometry (LC/MS) was used in 2 patients (5.7%). All patients had evidence of a clonal plasma cell dyscrasia by bone marrow biopsy and peripheral blood testing. Of the patients without subtyping, 8 (50%) were diagnosed before 2008.

DISCUSSION & CONCLUSIONS: Misdiagnosis of amyloidosis due to a lack of appropriate subtyping is a well-described and ongoing problem for patients with amyloidosis. These data suggest that definitive subtyping is still not routinely performed in the evaluation of amyloidosis. At our center, efforts to standardize the evaluation of Congo-red positive biopsies using definitive typing are underway.

REFERENCES:
Histopathology at the National Amyloidosis Centre: A 5 year review

NA Botcher¹, JA Gilbertson¹, JD Gillmore¹, PN Hawkins¹, A Wechalekar¹, HJ Lachmann¹

¹National Amyloidosis Centre, Division of Medicine, Royal Free Campus, UCL, Rowland Hill Street, London, NW3 2PF, UK.
n.botcher@ucl.ac.uk

Introduction
Accurate identification of amyloid type is critical in every case of systemic amyloidosis since therapy is type-specific. We present the findings of the National Amyloidosis Centre (NAC) Histopathological service highlighting some of the difficulties encountered.

Materials & Methods
The number of specimens received between 2011 and 2015 was identified and detailed results of the histopathological findings for those 5 years were reported. The tissue type was recorded and biopsies were stained with Congo red using the method of Puchtler et al [1]. Biopsies in which amyloid deposits were identified by Congo red were subsequently stained with a panel of antibodies against known amyloid fibril proteins. Mass spectrometry was introduced as a new method during this period for cases that were difficult to type using immunohistochemistry (IHC) alone. In these cases, amyloid was isolated from biopsies using laser capture microdissection and then analysed by mass spectrometry to determine which precursor proteins were present in the samples.

Results
6916 cases and over sixty different types of tissue were received at the NAC between 2011 and 2015. Material was received an average (median) of 6 days after being requested (range 1 – 961 days) and 460 requested biopsies were never received. Bone marrow trephines were the most commonly examined tissue (20%), followed by renal biopsies (18.4%), fat aspirates (17.8%) and gastrointestinal and cardiac biopsies (6% each). The majority of fat aspirates were harvested in clinic at the NAC and over the review period, and the number of fat aspirates collected more than tripled. Referring centres had a 22% false positive rate for amyloid and an 11% false negative rate. Of all biopsies examined, 37% contained no amyloid. Among amyloidotic biopsies, IHC definitively identified the amyloid type in 66% of cases, comprising AL in 45%, AA in 6% and TTR in 14%; all other positively identified types amounting to 2% of all the total. Among those with diagnostic light chain staining, 82% were lambda and 18% were kappa. There was no immunospecific staining in 27% of amyloidotic cases and 8% of biopsies were inadequate for IHC. 1740 samples were tested by mass spectrometry.

Discussion & Conclusions
Immunohistochemical typing and even routine Congo red histology in amyloidosis remain challenging. Specialist review at the NAC, where correct Congo red staining and specialised IHC can be complimented with proteomic analysis, should continue to be considered for the accurate identification of amyloid types.

References

Exercise capacity in Amyloidosis assessed by cardiopulmonary exercise testing

DL Brunjes¹, MS Maurer¹

¹Department of Medicine, Columbia University Medical Center, New York, NY, USA.
Dlt2127@cumc.columbia.edu

INTRODUCTION: Exercise intolerance is among the most common complaints expressed by heart failure (HF) patients. Functional decline compounds HF risks and results in diminished quality of life, increased frailty and higher mortality. Cardiopulmonary exercise testing (CPET) is often used to access prognosis in HF populations, but is also used as a marker of exercise intolerance. To date, only one study has examined CPET in cardiac amyloidosis and found that in patients with AL a VO₂ > 15 ml/kg/min is associated with greater survival. The main objective of this analysis is to compare exercise capacity in patients with AL and TTR amyloid. MATERIAL & METHODS: In addition to baseline characteristics, we collected peak VO₂, VO₂ at anaerobic threshold (AT), peak pulmonary ventilation (VE), respiratory exchange ratio (RER), and ventilator equivalent ratio for carbon dioxide (VE/VCO₂) from the CPET. RESULTS: Outcomes of CPET data for 45 patients with cardiac amyloidosis (26 AL and 19 TTR) were analysed. The AL subjects (age: 53±8, 16 males, 75±12kg) had an ejection fraction of 49±14% and the TTR subjects (age: 62±8, 16 males, 83±15kg) had an EF of 44±17%. As shown in the table below, peak VO₂, peak VE, and VO₂ at AT were not significantly different between groups, while RER was higher in the AL subjects and VE/VCO₂ showed a trend towards being higher in the TTR subjects. DISCUSSION & CONCLUSIONS: Measurements of oxygen consumption at peak and anaerobic threshold are not different between AL and TTR subjects, but the maximal effort ratio of carbon dioxide production to oxygen utilization (RER) is greater in AL subjects. Further research is needed to compare patients with cardiac amyloid to non-amyloid HFpEF patients and examine peripheral abnormalities associated with exercise intolerance.


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<th>AL (n=26)</th>
<th>TTR (n=19)</th>
<th>p-value</th>
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<td>Peak VO₂ (ml/kg/min)</td>
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<td>RER</td>
<td>1.19 ± 0.14</td>
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Multiple nodular pulmonary and subcutaneous amyloidosis associated with Sjögren syndrome

Y Kinoshita¹, Y Misumi¹, M Ueda¹, M Tasaki¹, T Masuda¹, G Suenaga¹, Y Inoue¹, K Obayashi², T Yamashita¹, Y Ando¹

¹Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan, ²Department of Morphological and Physiological Sciences, Graduate School of Health Sciences, Kumamoto University, Kumamoto, Japan

iidx.lily.lv@gmail.com

INTRODUCTION
Sjögren syndrome is a chronic autoimmune disease in which lymphocytes destroy the exocrine glands, specifically the salivary and lacrimal glands. Some patients with Sjögren syndrome also suffer from extraglandular disorders, including the skin, lung, heart, kidney, and nerve impairment. Sjögren syndrome is associated with lymphoproliferative disease in 7% of cases, and close relationship between chronic activation or dysregulation of the B lymphocytes and malignant transformation has been inferred. However the relationship between Sjögren syndrome and AL amyloidosis is unclear. We report a case with Sjögren syndrome and multiple lesions of nodular pulmonary and subcutaneous amyloidosis.

METHODS
We investigated clinicopathological characteristics of a 67-years-old woman with nodular pulmonary and subcutaneous amyloidosis associated with Sjögren syndrome.

RESULTS
Present case had a 5 year history of sicca syndrome, and she developed multiple subcutaneous and oral transmucosal nodules. Chest CT scan showed multiple nodular lesions in the bilateral lungs. Histopathological analyses of biopsied tissues from nodular lesions revealed massive deposition of AL amyloid fibrils. Serum immunoelectrophoretic study showed a monoclonal IgG λ-gammopathy. The κ/λ ratio was elevated with elevated level of both free κ and λ chains. A bone-marrow examination was within normal study. LC-MS/MS analyses of extracted amyloid fibrils detected IgG λ from pulmonary and oral transmucosal lesions, and IgG κ from a subcutaneous lesion. Manifestation of other organs including the heart, kidneys, GI tract, and nerves was not detected.

DISCUSSION & CONCLUSIONS
We present a very rare case with multiple nodular pulmonary and subcutaneous amyloidosis with Sjögren syndrome. The present case showed clinical characteristics different from primary AL amyloidosis in involved organs, clinical course, and biochemical features.
PC37

Associations between health-related quality of life and mortality in patients with AL amyloidosis by receipt of treatment regimens

V Sanchorawala1, S Lo1, MK White2, KL McCausland2, M Bayliss2, M Skinner1

1 Amyloidosis Center, Boston University School of Medicine and Boston Medical Center, Boston, Massachusetts, United States. 2 Optum, Lincoln, Rhode Island, United States

Vaishali.Sanchorawala@bmc.org

INTRODUCTION: Prior research shows that high-dose melphalan and stem cell transplantation (HDM/SCT) leads to improvement in health-related quality of life (HRQoL) as measured by the SF-36v1® Health Survey (SF-36v1) in patients with AL amyloidosis with greater SF-36v1 scores pre and post-treatment are associated with reduced risk of mortality. Here, we report HRQoL burden of AL amyloidosis and the association between baseline HRQoL and risk of 1-year and 2-year mortality after treatment with SCT and standard chemotherapy treatment regimens without SCT for AL amyloidosis.

METHODS: A modified version of the SF-36v1 was administered to all patients with AL amyloidosis evaluated at the Amyloidosis Center at Boston University between 1994 and 2014 (n=1,822). Analytic samples were created of all patients who received SCT (n=460) and of all patients who received standard chemotherapy without SCT (n=225). For both samples, patients completed the SF-36v1 prior to their first documented treatment regimen. The SF-36v1 assessed HRQoL across eight domains and two component summary measures: physical functioning (PF); role limitations due to physical health problems (RP); bodily pain (BP); general health (GH); vitality (VT); social functioning (SF); role limitations due to emotional health problems (RE); mental health (MH) and physical (PCS) and mental (MCS) component summaries. Analysis of variance was used to compare the norm-based SF-36v1 scores from all patients and the two analytic samples to a general U.S. population (USP). The USP data were adjusted to the age and gender distribution of the clinical samples using regression models, with each SF-36v1 domain scale or summary score as a dependent variable. Cox proportional hazard models were fit to examine the association of HRQoL and mortality at 1 and 2 years after starting treatment for both analytic samples. All models adjusted for age at baseline, gender, organ involvement (cardiac, renal, gastrointestinal, and hepatic), time from diagnosis to evaluation, and baseline HRQoL.

RESULTS: HRQoL of patients with AL amyloidosis is significantly worse across all eight SF-36v1 domain scales and summary measures as compared to a USP (p < 0.05 for all). The largest deficit was in PCS, where the mean scores among patients with AL amyloidosis was a full standard deviation worse than the USP (38.1 vs. 52.8, respectively, Cohen's d -0.791; p < 0.001). Greater HRQoL, as measured by baseline PCS, was associated with a reduced risk of 1-year (HR=0.94, 95% CI 0.92-0.96) and 2-year (HR=0.95, 95% CI 0.93-0.97) mortality among patients who received SCT. Similar associations were observed within the group treated with standard chemotherapy regimens without SCT; PCS scores were inversely associated with the risk of mortality during one year of follow-up (HR=0.97, 95% 0.94-0.997). These findings also persisted with an additional year of follow-up.

CONCLUSIONS: These results corroborate a previous relationship between HRQoL and mortality described by Seldin et al. after HDM/SCT. Moreover, these findings support a significant inverse association between HRQoL and risk of disease related mortality at 1 and 2 years following initiation of treatment for AL Amyloidosis, namely SCT and standard chemotherapy without SCT.


This research was supported by Amyloid research fund at BUSM and Prothena Biosciences Inc.
PC38

The role of induction therapy pre-autologous stem cell transplantation in patients with immunoglobulin light chain amyloidosis: a retrospective evaluation

YL Hwa, SK Kumar, MA Gertz, MQ Lacy, FK Buadi, TV Kouralis, WI Gonsalves, SV Rajkumar, RS Go, N Leung, PK Kapoor, D Dingli, RA Kyle, S Russell, JA Lust, SR Hayman, Y Lin, S Zeldenrust and AD Dispenzieri

Division of Hematology, Rochester Minnesota, USA
dispensieri.angela@mayo.edu

Introduction: There is no consensus about whether patients with immunoglobulin light chain amyloidosis (AL) should receive induction therapy prior to autologous stem cell transplant (ASCT). The aim of this study was to investigate the relationships between baseline bone marrow plasmacytosis (BMPC), cardiac staging, and pre-transplant induction chemotherapy among patients with AL.

Methods: All patients who received ASCT for AL within 12 months of their diagnosis were included. Patient characteristics and outcomes were abstracted. Univariate and multivariate modeling was performed.

Results: Among the 415 AL patients, 33% had induction prior to ASCT. Post-ASCT hematologic CR plus VGPR rates were significantly higher in the patients with baseline BMPC ≤ 10% compared to BMPC >10% (58% versus 40%, p=0.0013). Significant risk factors for lack of attainment of CR included attenuated dose melphalan conditioning, baseline BMPC>10%, no induction therapy, and male gender. Five-year OS for the entire group was 65%. The multivariate model for inferior OS included no induction therapy, advanced AL amyloid staging, BMPC>10%, attenuated conditioning melphalan dose, and male gender. We next explored whether there was a subset of patients who could do extremely well even without induction, specifically the Mayo 2012 stage I-II patients with BMPC≤10%. This group comprised 56% of the population, 43% of whom received no induction and 13% of whom received induction. The 5-year OS of these low-risk populations were 81% and 83%, respectively.

Conclusions: Induction therapy pre-ASCT may improve outcomes among patients with AL amyloidosis. Whether all patients should receive induction is unclear, since low-risk patients appear to do extremely well without induction therapy. Prospective study will be required.
PC39

Pomalidomide and dexamethasone in patients with relapsed AL amyloidosis: results of a phase I/II study

V Sanchorawala, A Shelton, S Lo, JM Sloan, C Varga, DC Seldin

Amyloidosis Center, Boston University School of Medicine, Boston, MA, USA
vaishali.sanchorawala@bmc.org

INTRODUCTION: Immunomodulatory agent, lenalidomide, has been shown to induce hematologic response and improve survival in patients with relapsed AL amyloidosis. Pomalidomide, a third generation immunomodulatory agent, was studied in combination with low dose dexamethasone in a prospective phase I/II study of patients with relapsed AL amyloidosis (Clinicaltrials.gov identifier NCT01570387).

METHODS: Eligibility criteria included: age ≥18 years, relapsed AL amyloidosis after ≥1 prior therapy, measurable plasma cell dyscrasia with dFLC of > 50 mg/L (difference between involved and uninvolved free light chain levels), involvement of at least one major vital organ and serum creatinine < 3.0 mg/dL. Patients received pomalidomide in a standard 3+3 dose escalation design on days 1-28 of a 28-day cycle for cohort 1 (2 mg) and 2 (3 mg), and on days 1-21 of a 28-day cycle for cohort 3 (4 mg). Low dose dexamethasone was administered at 20 mg orally once a week. All patients received prophylaxis with daily aspirin and a proton pump inhibitor. MTD was defined as the highest dose resulting in DLT during cycle 1 in < 1 of 6 patients. Hematologic and organ responses were measured after 3 cycles. Treatment was continued until progression, toxicity or achievement of a hematologic complete response. An expansion cohort of 12 patients was enrolled at the MTD. The objectives were to determine the safety, tolerability, MTD, and recommended phase II dose of the drug, as well as to evaluate hematologic and clinical response.

RESULTS: Twenty-seven patients with relapsed AL amyloidosis have been enrolled from June 2012 to August 2015; 15 in phase I and 12 in the expansion phase II cohort. The median age was 68 years (range, 44-79), and median number of prior therapies was 2 (range, 1-6): 16 (59%) had received HDM/SCT, 13 (48%) had received prior lenalidomide, and 21 (78%) had received bortezomib. The median time from diagnosis to enrollment on this trial was 27 months (range, 4-246) and the median time from last treatment to enrollment was 5 months (range, 1-89). The median number of organ system involvement was 2 (range, 1-6). Seventeen patients (63%) had ≥2 organs involved, 14 patients with renal and 17 patients with cardiac involvement. Twenty-three (85%) patients had cardiac biomarker stage II or III disease. Six patients were treated in the 2 mg cohort (this cohort was expanded due to grade 3 renal failure in cycle 3 among one of the first 3 patients) and 3 were treated in the 3 mg and 6 in the 4 mg cohort (this cohort was expanded due to grade 3 pneumonia with neutropenia in cycle 1, considered as a DLT). There were 2 on-study deaths due to GI bleeding/sepsis and Legionella pneumonia/sepsis, respectively. Twenty-four patients have discontinued study treatment due to disease progression (n=8), withdrawal by patient (n=5), hematologic CR (n=7), investigator’s choice (n=1), and death (n=3). Median number of cycles administered is 6 (range, 0-18) and 3 patients continue on therapy as of data cut-off of Feb 29, 2016. Twenty patients (74%) experienced at least one grade ≥3 AE (any cause). The most common drug-related AEs included: fatigue (63%), neutropenia (63%), anemia (52%), and thrombocytopenia (30%). There was a paradoxical increase of BNP by 30% in 20 patients (74%) after 1 cycle and in 26 patients (96%) after the first 3 cycles of therapy. Median time to best hematologic response was 3 months (range, 3-9) and median duration of hematologic CR after discontinuation of pomalidomide was 15 months (range, 3-26).

Table 1: Hematologic response

<table>
<thead>
<tr>
<th></th>
<th>Phase I (%)</th>
<th>Phase II (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluable patients*</td>
<td>(n=14)</td>
<td>(n=10)</td>
<td>(n=24)</td>
</tr>
<tr>
<td>CR</td>
<td>4 (28.6%)</td>
<td>4 (40.0%)</td>
<td>8 (33.3%)</td>
</tr>
<tr>
<td>VGPR</td>
<td>0 (0.0%)</td>
<td>1 (10.0%)</td>
<td>1 (4.2%)</td>
</tr>
<tr>
<td>PR</td>
<td>2 (14.3%)</td>
<td>1 (10.0%)</td>
<td>3 (12.5%)</td>
</tr>
<tr>
<td>Response ≥ VGPR</td>
<td>4 (28.6%)</td>
<td>5 (50.0%)</td>
<td>9 (37.5%)</td>
</tr>
</tbody>
</table>

*Evaluable patients defined as those completing ≥ 3 cycles of treatment

CONCLUSIONS: Pomalidomide and dexamethasone treatment is feasible in patients with relapsed AL amyloidosis, with MTD defined as 4 mg/day Days 1-21 of a 28 day cycle. Hematologic response of ≥ PR occurred in 50% of patients.

This clinical trial was partly supported by Celgene Corporation.
Validation of new renal staging in AL amyloidosis treated with high dose melphalan and stem cell transplantation

A Havasi1,2, L Stern1,2, F Sun2,3, V Sanchorawala2,4

1Department of Nephrology, Boston University School of Medicine, Boston, MA. 2Amyloidosis Center, Boston University School of Medicine. 3Department of Biostatistics, Boston University School of Public Health, Boston, MA. 4Section of Hematology-Oncology, Boston University School of Medicine, Boston, MA. Email: Vaishali.Sanchorawala@bmc.org

INTRODUCTION: Simple and validated criteria for baseline staging of renal disease associated with AL amyloidosis have been proposed in a recent analysis by Palladini et al1. It is imperative to predict the course of progression to end stage renal disease (ESRD), as this markedly increases morbidity and leads to treatment limitations. The proposed staging system based on universally available measurements (i.e. proteinuria and eGFR) sharply discriminated three groups of patients with different probabilities of reaching ESRD. However, in this study all patients with new diagnosis of AL amyloidosis were included regardless of their treatment modality and only 13.8% (Pavia) and 29.5% (Heidelberg) underwent high dose melphalan and stem cell transplantation (HDM/SCT). Here, we report on performance of this new renal staging system in patients with AL amyloidosis treated with HDM/SCT.

MATERIAL & METHODS: Total of 421 consecutive patients with AL amyloidosis treated with HDM/SCT at the Amyloidosis Center from July 1994 to December 2008 were included. Baseline patient characteristics are detailed in Cibeira et al2. Kaplan-Meier plots were used in survival analysis.

RESULTS: In this cohort, 332 patients (78%) had renal involvement according to the consensus criteria of ISA published in 2005. Thirty-two patients who were on dialysis at the time of SCT were excluded. A total of 59 (19%) patients required dialysis. Renal survival was significantly longer in our cohort of HDM/SCT (Fig. 1A). Median renal survival was 13.4 years with a median follow-up of 10.7 years. The largest proportion of patients (52%) had stage II disease, mainly due to meeting the criteria of proteinuria. Progression to dialysis and 2-year dialysis risk according to renal stage are shown in figure 1B and 1C.

DISCUSSION & CONCLUSIONS: Renal survival is much improved when treated with HDM/SCT. The new renal staging system for prediction of progression to ESRD is validated in patients with AL amyloidosis undergoing HDM/SCT as well, however, with less precision. It does not discriminate well between stage I and stage II patients in the first 2 years after HDM/SCT.


This research was supported by Amyloid Research Fund at Boston University School of Medicine.
PC41

Heavy light chain test quantifies clonal plasma cell disease in patients with AL amyloidosis and low serum free light chain burden

T Prokaeva1, B Spencer1, F Sun2, N McConnell3, RM O’Hara4, DC Seldin1, LH Connors3 and V Sanchorawala1

Boston University 1Amyloidosis Center and 2School of Public Health, Boston, MA; 3The Binding Site Group Ltd., Birmingham, United Kingdom; 4The Binding Site, Inc., San Diego, CA; prokaeva@bu.edu

INTRODUCTION: Serum and urine immunofixation electrophoreses (SIFE/UIFE) are routinely used for detection of clonal immunoglobulins in AL amyloidosis. The Freelite® assay has significantly improved the care of patients by providing quantitative measure for the detection and monitoring of clonal disease. However, 13% to 25% of patients may have uninformative serum free light chain (sFLC) values making it difficult to reliably diagnose clonality and measure the hematologic response. In addition, patients with low sFLC burden (dFLC<50 mg/L) are frequently excluded from clinical trials. Recently, the Hevylite® assay which targets junctional epitopes between LCs bound to heavy chain partners was developed. The objective of this study was to assess the diagnostic and quantitative potential of serum heavy light chain (HLC) measurements in patients with AL amyloidosis.

MATERIAL & METHODS: Untreated patients with AL amyloidosis in the absence of multiple myeloma or B cell lymphoproliferative diseases (n=199) were included in this study. Serum samples were obtained and SIFE/UIFE performed at initial evaluation. HLC pairs were evaluated by Hevylite® assay and HLC κ/λ ratios (HLCr) were calculated. An HLCr abnormality was defined as a ratio outside of the reference range as a result of one HLC pair concentration increased above or decreased below the reference range. sFLCs were assessed with the Freelite® assay. dFLC was defined as the difference between involved and uninvolved sFLC.

RESULTS: An abnormal HLCr was present in 74 (37.2%), an abnormal FLCr in 163 (81.9%), and SIFE/UIFE (+) in 187 (94%) of patients. Seventy-two cases presented with an abnormal HLCr in a single Ig isotype. In all cases, the LC isotype of the involved HLC was consistent with the LC restriction identified by other tests. In 69 cases, Ig isotype of the abnormal HLCr matched SIFE/UIFE results; 4 cases identified as FLC by other tests were found to have an HLCr abnormality; and in 1 bi-clonal IgA+IgM case, an additional abnormal IgG HLCr was identified. In total, 186 (93.5%) patients had abnormalities in either HLCr or FLCr. SIFE/UIFE combined with each test identified clonality in 187 (94%) and 199 (100%) patients, respectively. None of 12 SIFE/UIFE (-) cases presented with an abnormal HLCr; however, all showed abnormal FLCr. Cases with normal FLCr had HLCr abnormality more often compared to those with abnormal FLCr (63.9% vs. 31.3%; P = 0.001). The risk of HLCr abnormality was significantly associated with normal FLCr (OR = 3.9; P = 0.0004). HLCr was not a predictor of overall survival (data not shown). HLCr abnormality was found in 26 (70.3%) of 37 patients with dFLC<50 mg/L vs. 48 (29.6%) of 162 patients with dFLC≥50 mg/L (P<0.05). Median abnormal values and percent of HLCr abnormality in 26 patients with dFLC < 50 mg/L are shown in the table.

<table>
<thead>
<tr>
<th>Abnormal HLCr isotype</th>
<th>n</th>
<th>Abnormal FLCr, n</th>
<th>HLCr, median (range)</th>
<th>% HLCr value above/below normal range, median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgGλ</td>
<td>15</td>
<td>3</td>
<td>0.26 (0.09-0.93)</td>
<td>78.8 (16.7-84.9)</td>
</tr>
<tr>
<td>IgGκ</td>
<td>1</td>
<td>1</td>
<td>3.54</td>
<td>10.2</td>
</tr>
<tr>
<td>IgAλ</td>
<td>6</td>
<td>2</td>
<td>0.05 (0.001-0.69)</td>
<td>93.4 (11.4-99.9)</td>
</tr>
<tr>
<td>IgAκ</td>
<td>1</td>
<td>1</td>
<td>13.78</td>
<td>610.1</td>
</tr>
<tr>
<td>IgMλ</td>
<td>1</td>
<td>1</td>
<td>0.04</td>
<td>96.3</td>
</tr>
<tr>
<td>IgMκ</td>
<td>1</td>
<td>1</td>
<td>36.99</td>
<td>1250.1</td>
</tr>
<tr>
<td>IgG+IgA+IgM</td>
<td>1</td>
<td>-</td>
<td>0.62; 0.92; 0.74</td>
<td>20.6; 18.2; 37.4</td>
</tr>
</tbody>
</table>

Normal HLCr ranges: IgG κ/λ, 1.12-3.2; IgA κ/λ, 0.78-1.94; and IgM κ/λ, 1.18-2.74.

DISCUSSION & CONCLUSIONS: In our series, HLC test was found to be useful for quantification of plasma cell clonality in patients with AL amyloidosis and dFLC<50 mg/L. FLCr demonstrated superior sensitivity for identification of plasma cell clonality when combined with SIFE/UIFE, however, we noted a high proportion of cases with abnormal HLCr in the group with normal FLCr. These findings suggest that the Hevylite® assay has potential as a supplemental test for measuring and monitoring clonality in patients with low FLC burden.

This research was supported by the Boston University Amyloid Research Fund, Wildflower Foundation and Gruss Foundation.
The first detailed postmortem pathological study of an AH amyloidosis patient who survived 17 years after the onset without any specific chemotherapies

M Yazaki1,2, K Ueno1, N Katoh1, T Yoshinaga1, Y Sekijima1,2, S Ichimata3, M Kobayashi3, H Kanno3, S-I Ikeda1,2

1 Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan.
2 Institute for Biomedical Sciences, Shinshu University, Matsumoto, Japan.
3 Department of Pathology, Shinshu University School of Medicine, Matsumoto, Japan

mayazaki@shinshu-u.ac.jp

INTRODUCTION

AH amyloidosis, resulting from the deposition of amyloid fibrils derived from immunoglobulin (Ig) heavy chain, is still recognized as a rare disorder [1]. While it is well-known that nephropathy is the main clinical manifestations in the AH amyloidosis patients, the precise pathological features of this disease has not been fully understood. Here, we first report the detailed histopathological characteristics of an autopsied patient with AH amyloidosis. This patient survived long after the onset (17 years) without any specific treatments except for hemodialysis.

MATERIAL & METHODS

The patient was an 83-year-old female with monoclonal gammopathy (λIgG). At age 66, anemia was detected and renal dysfunction was revealed at age of 68. Renal biopsy showed heavy glomerular amyloid deposits and the diagnosis of AH amyloidosis was made by biochemical analysis of the deposited amyloid fibrils, composed of variable region fragments of Ig heavy chain [2]. Bone marrow examination demonstrated plasma cell dyscrasia. Clinically, there were no overt signs of amyloid cardiomyopathy, neuropathy, or gastroenteropathy. She denied a specific chemotherapy and her renal functions slowly deteriorated. Hemodialysis was started on age 75 years. Her physical condition had been stable by 81 year-old, but her condition gradually deteriorated with ageing. At age of 83, she died of worsening of systemic condition due to renal insufficiency and autopsy was performed.

RESULTS

Heavy amyloid deposition was seen especially on renal glomeruli. In addition, marked amyloid deposits were observed peripheral and autonomic nerve systems, although the patient did not have symptoms associated with neuropathy. On the other hand, only mild amyloid deposits were shown in the liver, cardiac muscles, tongue, GI tract, and lung. In the central nervous system, amyloid deposits were seen on the small vessel walls of choroid plexus in the brain, but no amyloid associated with heavy chain was detected in the parenchyma of the brain.

DISCUSSION & CONCLUSIONS

The amyloid selectively affected some systemic organs, especially kidney, and amyloid deposits were seen more remarkably on small vessel walls of systemic organs rather than on large vessels. Since involvements of the heart, GI tract, and liver were not so significant, this patient could survive long after the onset despite no any specific therapies.

REFERENCES

End-stage renal failure due to transthyretin amyloidosis after liver transplantation: outcomes in 19 registry cases

A Rocha1,2, I Beirão1,2,3, H Pessegueiro4, R Almeida5, L Lobato1,2,3

1Unidade Corino de Andrade, Porto, Portugal; 2Unit for Multidisciplinary Research in Biomedicine-UMIB, Instituto de Ciências Biomédicas Abel Salazar-ICBAS, Porto University, Porto, Portugal; 3Department of Nephrology, Centro Hospitalar do Porto, Porto, Portugal; 4Department of Internal Medicine, Centro Hospitalar do Porto, Porto, Portugal. 5Department of Vascular Surgery, Centro Hospitalar do Porto, Porto, Portugal.

acrisbraga@gmail.com

INTRODUCTION: Transthyretin amyloidosis (ATTR) patients with the V30M mutation, who progress to end-stage renal disease (ESRD) are mostly female with later onset of neuropathy, usually after the sixth decade of life. Variable levels of albuminuria, progressive renal insufficiency and heavy amyloid infiltration of the kidney (1) characterize these patients. ESRD aggregates in families. Considering ESRD after liver transplantation (LT) in ATTR, a larger cohort of patients has never been analyzed.

The aim of this study was to provide epidemiological data and clinical characteristics of ATTR LT patients that progress towards dialysis.

MATERIAL & METHODS: Inclusion criteria were LT patients with the V30M mutation that start dialysis after the first year of grafting. The period of study comprises January 1997 to February 2015. The events were registered from the first day of dialysis to the study’s end point on February 29, 2016. Family records were reviewed. All patients were followed in the same center.

The admission criteria for LT comprises a GFR estimated by cystatin C and CKD-EPI formula > 60 mL/min at the time of liver surgery; immunosuppressive regimen was based in calcineurinic inhibitors (CI) and mofetil mycophenolate; whenever possible CI was suspended.

RESULTS: We registered 12 males and 7 females, belonging to 18 families. Median age at the onset of neuropathy was 32 years with a median evolution of disease at LT of 5 years. One fourth of patients augmented proteinuria after LT. Renal biopsy was performed in 4 patients before LT, all specimens have amyloid; in one, biopsy was repeated 3 years after LT revealing more extensive amyloid deposits. Hemodialysis was the treatment of choice in all, implemented 8 ± 5 years after surgery; 7 patients required urgent initiation of dialysis. Three patients were lost for follow-up. Four patients received a kidney transplant (KT) after a median of 12 months on dialysis. Median follow-up after KT was 8.5 years. One patient lost KT function after 11 years and died 5 years after resumed dialysis, other died 10 years after KT. Among the 12 patients who remained on dialysis, 50% died after a median of 2.2 years. The median survival on dialysis was 2 years. The causes of death were cardiovascular events (50%) and infectious diseases (50%). The pedigree analysis showed that in 9 cases (47%), at least 1 relative (non-LT) progressed to ESRD, in 4 of them the transmitting parent.

DISCUSSION & CONCLUSIONS: In liver transplant patients, on contrary to classical nephropathy in ATTR V30M, ESRD predominates in men, but familial aggregation of renal disease is conserved. Kidney amyloidosis can progress after LT. The type of fibrils deposited in the kidney after transplantation, can lead men to lose their protection of renal disease. Dialysis dependence is associated with elevated mortality in LT patients.

OUTCOME AFTER LIVER TRANSPLANTATION FOR TRANSTHYRETIN FAMILIAL AMYLOID POLYNEUROPATHY: ANALYSIS OF DEATH CAUSE AND TEMPORAL TRENDS

V Algalarrondo¹, Teresa Antonini², Marie Théaudin³, D Chemla⁴, A Benmalek⁵, Catherine Lacroix⁶, D Castaing², Cécile Cauquil³, Sylvie Dinanian¹, Ludivine Eliahou¹, F Rouzet⁶, Dominique Le Guludec⁶, M S Slama¹, D Samuel², D Adams³

(¹) Hopital Antoine Beclere, APHP, Cardiology Department, Universite Paris Sud, CRMR NNERF, Clamart, France
(²) Hopital Paul Brousse, Hepato-Biliary Center, AP-HP, Inserm U1193, Université Paris-Sud, CRMR NNERF, Villejuif, France. (3) Hopital Bicetre, APHP, Neurology, Universite Paris Sud, CRMR NNERF, Inserm U1195, Le Kremlin-Bicetre, France (4) Physiology Department, EA4533, Universite Paris-Sud, Le Kremlin Bicêtre, France (5) School of Pharmacy, Universite Paris-Sud, Chatenay Malabry, France (6) Hopital Bichat-Claude Bernard, APHP, Nuclear Medicine, Université Paris VII, Inserm U1148, Paris, France

prmslama@gmail.com

Background: Mutated transthyretin amyloidosis (m-aTTR) is a multisystemic disease involving both the heart and the peripheral nervous system. Liver transplantation (LT) is the reference treatment of m-aTTR familial amyloid polyneuropathy (FAP) and preoperative detection of high risk patients is crucial.

Objectives: 1) To characterize the temporal trends and the causes of death of m-aTTR FAP patients after LT and 2) To explore how these trends would affect risk prediction.

Methods: A retrospective longitudinal cohort study was performed on 215 consecutive m-aTTR FAP patients who underwent LT. Causes of death were analyzed and sorted into m-aTTR related mortality (cardiac events, end stage amyloidosis, stroke) and non m-aTTR related mortality (surgical and graft complications, infections). We analyzed how these trends would affect risk prediction in m-aTTR patients before LT using three predictive techniques (clinical risk score, heart rate response to atropine and ¹²³I-MIBG scintigraphy).

Results: Over a median follow up of 1439 patients-year (median: 5.9 years) after LT, 84 patients died. Rate of death was higher in the first year following LT than thereafter (13.0 vs. 4.3±1.8%/year; P=0.004). Cardiac events ranked as the first leading cause of death (38%), followed by infections (24%), graft complications (17%), end stage amyloidosis, stroke and miscellaneous (7% each). Deaths due to infections and graft complications occurred significantly earlier than those due to end stage amyloidosis and stroke. M-aTTR related mortality was associated with the severity of cardiac amyloidosis and neuropathy. Conversely, non m-aTTR related mortality was associated with perioperative period related variables and thus, blunted significantly the accuracy of the predictive risk scores based on preoperative evaluation.

Conclusions: In m-aTTR FAP, causes of death after liver transplantation followed significant temporal trends. The leading cause of death was cardiac events. Close cardiac and neurological preoperative evaluation allows to predict m-aTTR FAP related mortality with accuracy.
Orthotopic heart transplantation in a 65 year old patient with Trans Thyretin Amyloidosis with de-novo Pro24Ser Mutation
Amarinder Bindra (1), Merrill Benson (2), Shelley Hall(1)

(1) Baylor University Medical Center, Dallas, Texas
(2) Indiana University, Indianapolis, Indiana

We present a case of de-novo Pro24Ser mutation who presented to our institute with the diagnosis of hypertrophic cardiomyopathy. He is a 65 year old Caucasian male who had been extremely symptomatic from heart failure symptoms for the past few years. History and imaging data were reviewed and an infiltrative cause for heart failure was suspected. A diagnosis of TTR Amyloidosis was made with aid of myocardial biopsy. We isolated DNA from the heart tissue and confirmed the Pro24Ser mutation. A tryptic digest of the isolated protein gave peptides with TTR sequence. The significant tryptic peptides were residues 22-34 with the normal (wild type) sequence starting at a.a. residue 22- GSPAINVAVHVFR representing 44% of the protein and the variant sequence GSSAINVAVHVFR representing 56% of the protein. There was no evidence of systemic Amyloidosis in this patient, thus the patient underwent a successful Orthotopic heart transplant in September of 2015. Patient’s brother and daughter were screened for the mutation and were found to be negative for the same.

Pro24S mutation was detected in humans in 1995 and ours is the first known patient to have undergone a successful heart transplantation. The patient is followed regularly in our clinic and has not had any evidence of recurrence of cardiac amyloidosis so far. He is not on any disease modifying medications except routine immunosuppressants post transplantation.

Short axis and long axis echocardiogram images showing massive infiltration of the left ventricle of the heart with Amyloidotic protein
Monitoring recurrence of cardiac amyloidosis following heart transplantation due to immunoglobulin light-chain (AL) amyloidosis

M. Solé1, MT. Cibeira2, JT Ortiz3, M. Rovira2, C. Fernández de Larrea2, JI Aróstegui4, X Bosch3, L. Rosiñol2, F Pérez-Villa4, Joan Bladé2

1 Department of Pathology. 2 Department of Hematology. 3 Department of Cardiology. 4 Department of Immunology, Amyloidosis and Myeloma Unit, Hospital Clinic, Institut d’Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, Barcelona, Spain.

mcibeira@clinic.ub.es

INTRODUCTION: Immunoglobulin light chain (AL) amyloidosis is the most prevalent type of systemic amyloidosis in western countries. Organ damage is produced by intercellular deposition of amyloid as well as by direct toxicity of circulating amyloidogenic light chains. Prognosis depends on tumour burden and, most importantly, on the presence and severity of cardiac involvement. Patients with advanced amyloid cardiomyopathy (ACM) of AL type have a very poor outcome. Heart transplantation (HT) followed by high-dose melphalan and autologous stem cell transplant (ASCT) should be considered in a selected group of these patients with isolated or predominant severe cardiac involvement.

MATERIAL & METHODS: We retrospectively analysed the series of patients undergoing heart transplantation (HT) at our institution for ACM of AL type between 2000 and 2014. Complete follow-up was available for all patients. Myocardial biopsies for monitoring of organ rejection were performed in all patients at different time points until one year beyond HT, according to institutional protocols. Monitoring by measurement of serum free light-chains (sFLC) was available at our centre from 2008.

RESULTS: Twelve patients with ACM underwent HT during the study period, accounting for 4% of all HT performed at our institution. Amyloid subtype was AL in 5 patients and transthyretin (ATTR) in 7, including 6 mutated and 1 wild-type ATTR. Regarding the 5 patients with ACM of AL type, 4 of them were women and the median age was 50 years (range, 43-56). Light-chain isotype was lambda in all of them, with a median difference between involved and uninvolved sFLC of 207.5 mg/L (range, 26-348) and median bone marrow infiltration by 11% plasma cells (range, 1-24). Extracardiac involvement was present in 4 patients but was clinically relevant in only one of them, who achieved a renal and liver response after chemotherapy but had progressive heart failure leading to HT at 18 months after initial diagnosis. Three of the 5 patients received prior chemotherapy and two achieved hematologic response. Median time from diagnosis of AL amyloidosis to HT was 5 months. Three patients underwent an ASCT at a median time from HT of 6 months and all of them achieved a hematologic complete response (CR) sustained during 6 years, 3 years and 10 months after ASCT. Endomyocardial biopsies of transplanted heart did not show amyloid deposits in two patients who obtained hematologic response after initial chemotherapy while showed focal vascular amyloid infiltration (at 12 months post-HT) in the patient who did not respond. Myocardial biopsies of the patient who did not receive prior chemotherapy nor autologous transplant showed focal amyloid deposits at 12 months and diffuse infiltration at 14 months after HT, and the patient finally died at 22 months post-HT due to progressive cardiac and gastrointestinal amyloid involvement. The last patient achieved a PR after 2 cycles of chemotherapy, underwent HT complicated with end-stage renal failure and cytomegalovirus infection resistant to antiviral therapy, and has not been able to receive an ASCT yet. An endomyocardial biopsy performed 5 months after HT ruled out amyloid infiltration and the patient remains in stable PR despite no further chemotherapy, sixteen months after HT.

DISCUSSION & CONCLUSIONS: In our small series of patients with AL amyloidosis who have received a HT, recurrence of ACM in the graft was observed in two patients at 12 months after transplant. Recurrence was symptomatic and led to death in the only patient who did not receive chemotherapy, while it was subclinical in one patient who did not respond to initial chemotherapy and remained with disease activity until ASCT, probably allowing deposition in the donor heart. The latter patient achieved CR after ASCT and is free of signs and symptoms of ACM three years after ASCT. Our observations, suggest that effective chemotherapy should be started prior to HT and that ASCT should be performed as soon as possible, particularly in those patients without hematologic response.
Wild-type TTR amyloidosis: is there any role for heart transplantation?

MT Cibeira¹, JT Ortiz², M Solé³, JI Aróstegui¹, Carlos Fernández de Larrea¹, X Bosch², F Pérez-Villa², Joan Bladé¹

¹ Department of Hematology, ² Department of Cardiology, ³ Department of Pathology, ⁴ Department of Immunology, Amyloidosis and Myeloma Unit, Hospital Clinic, Institut d’Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, Barcelona, Spain.

mcibeira@clinic.ub.es

INTRODUCTION: Wild-type transthyretin amyloidosis (ATTRwt), formerly known as senile systemic amyloidosis, is an aging-related disorder characterized by deposition of amyloid fibrils in the heart. Patients are more frequently males and may present with congestive heart failure and atrial fibrillation, frequently preceded by carpal tunnel syndrome. Diagnosis requires demonstration of amyloid deposition in a heart biopsy and positivity of amyloid typing for TTR. Also, TTR mutations associated with hereditary amyloidosis should be ruled out. However, heart biopsy is usually avoided in patients with advanced age and, consequently, postmortem studies suggest that prevalence of ATTRwt is underestimated. While awaiting for specific anti-amyloid therapy, treatment of ATTRwt consists in medical treatment of heart failure.

MATERIAL, METHODS & RESULTS: Herein we describe a 63 year old male who presented with heart failure symptoms from 3 years before. He received medical therapy, poorly tolerated due to hypotension. Electrocardiogram did not show low voltages. Echocardiography revealed a non-obstructive hypertrophic myocardiopathy, with an increased interventricular septum (19 mm), normal ejection fraction and diastolic dysfunction. A cardiac magnetic resonance confirmed these findings and the late gadolinium enhancement pattern suggested an infiltrative aetiology. A monoclonal gammopathy was then ruled out and lab tests showed increased cardiac biomarkers, with troponin I of 0.09 ng/mL (normal range <0.05) and brain natriuretic peptide (BNP) of 826 pg/mL (normal range <37). Fat pad aspiration and rectal biopsy failed to show amyloid deposits. A myocardial biopsy revealed massive deposition of amyloid (Congo red positive) and immunohistochemical staining showed TTR positivity in the amyloid deposits. No TTR mutations were found in genomic DNA analysis of the TTR exons. Accordingly, diagnosis of ATTRwt was established. The patient experienced a progressive worsening of cardiac functional class and ejection fraction, increase of BNP serum levels (up to 1274 pg/mL) and developed an atrioventricular block with sinusual bradycardia, requiring an implantable cardioverter defibrillator. At that time, the patient was included in the waiting list for heart transplant. Three months later, he was admitted due to requirement of inotropic therapy and intravenous diuretic support, and finally received a heart transplant on September 2010. Despite 3 episodes of mild acute rejection, the outcome was good. Last biopsy of donor heart was performed one year after transplant and did not show amyloid deposition. Five years later, the patient remains in good performance status under immunosuppressive therapy, with normal cardiac function and normal cardiac biomarkers.

DISCUSSION & CONCLUSIONS: Very few cases of heart transplantation in ATTRwt have been reported (1-3). Although this disease involves an aging population, median age being 75.6 years in a recent study (4), some patients are less than 65 years old. The rarity of extracardiac involvement, the slow amyloid deposition rate and the fact that autologous wild type TTR might not have the same appetite for deposition in the donor heart, makes heart transplant a treatment option in younger patients with severe cardiac involvement.

Heart transplantation: the last possibility for patients with amyloid AL cardiomyopathy

EM Nucifora, MA Aguirre, D Fantl, J Arbelbide, P Sorroche, N Schutz, C Belziti, B Boietti, D Perez de Arenaza, M S Saez, DH Giunta, ML Posadas Martinez

Department of Medicine, Instituto Universitario Hospital Italiano de Buenos Aires, Argentina. Grupo de Estudio de Amiloidosis,

elsa.nucifora@hospitalitaliano.org.ar

Background: Amyloid cardiomyopathy is the most serious complication of AL amyloidosis. The medical community is not always aware of the high mortality associated with this condition and the urgency to treat it.

Aim: To present the experience of a university hospital in Argentina the treatment of AL amyloidosis in patients with severe heart failure and cardiomyopathy due to AL amyloidosis.

Methods: Review of medical records of patients with AL amyloidosis, heart failure and cardiac transplantation.

Results: Case 1: Male, 38y. Rapidly progressive cardiac failure. He was admitted to the hospital with stage III NYHA. BNP: 2600, high serum free lambda light chain were present. Hemodynamic support was required and he underwent an emergency heart transplantation. Four months later, he received four cycles of CYBORD and autologous bone marrow transplantation. He is in complete remission after 90 months of follow up.

Case 2. 60y man. Rapidly progressive cardiac failure stage III NYHA. Cardiac ultrasound and MRI were compatible with amyloid cardiomyopathy. High lambda light chain serum were detected. He underwent a heart transplantation. He died three months after from urosepsis.

Case 3: Male 62Y. Newly diagnosed AL amyloidosis, high serum free lambda light chain were detected. He developed rapidly progressive heart failure stage III NYHA and underwent a heart transplantation. He started treatment with CYBORD after recovery of the heart transplant and is alive after 21 months follow up.

Case 4: Female 45y. Severe heart failure developed in 6 months. When she arrived at the hospital she needed urgent heart transplant. She developed atrial fibrillation, ischemic stroke and glomerular proteinuria. Once transplanted, he received CYBORD and autologous bone marrow transplantation. She is in complete remission after 7 months of follow up.

Summary: In Argentina, amyloidosis is under diagnosed. This is critical for patients with cardiac amyloidosis. Our experience in treating patients with stage III NYHA heart failure due to amyloid cardiomyopathy with initial heart transplantation followed by bortezomib based chemotherapy and autologous bone marrow transplantation is encouraging people considering the bad prognosis of this condition.

References:


Clinicopathological characterizations of transmitted transthyretin amyloidosis after domino liver transplantation: a single-center experience

T Yoshinaga1, M Yazaki2, Y Sekijima1,2, T. Ikegami3, S Miyagawa1, S Ikeda1,2

1Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan.
2Institute for Biomedical Sciences, Shinshu University, Matsumoto, Japan.
3Department of Surgery, Shinshu University School of Medicine, Matsumoto, Japan

kiccho828@gmail.com

INTRODUCTION

The most serious issue in domino liver transplantation (DLT) associated with transthyretin (TTR)-related familial amyloid polyneuropathy (FAP) is iatrogenic transmitted amyloidosis in DLT-recipients. However, clinicopathological features of this de novo amyloidosis and therapeutic strategy have not been fully understood. The aim of this study is to elucidate clinicopathological features of DLT-recipients and to establish the therapeutic strategy for the de novo amyloidosis.

MATERIAL & METHODS

Postoperative clinical courses and laboratory data were examined in 11 DLT-recipients (6 males), who have been followed-up at Shinshu University Hospital. The types of TTR variant of FAP donors were an ATTR V30M (10) and a V30L (1). The mean age of recipients at DLT was 53.9 ± 10.2 years (32 to 65 years). The amyloid deposits were monitored by serial gastroduodenal biopsies. We re-evaluated the presence of amyloid deposits on biopsied samples after preparation of 100 consecutive tissue-sections, previously judged as “no amyloid deposits” in routine histopathological examination. The majority of recipients were started on oral intake TTR-stabilizers (diflunisal or tafamidis) after detection of amyloid deposition. One recipient with an FAP (V30L) liver began to take diflunisal from asymptomatic stage 8 years before detection of amyloid, since the FAP donor died of worsening of cardiomyopathy one year after DLT [1].

RESULTS

Of 11 recipients, three developed clinical symptoms associated with sensory polyneuropathy in lower limbs which started from 8 postoperative years. No one has presented with autonomic neuropathy, oculopathy, or cardiomyopathy. Nerve conduction studies revealed that CMAP (compound muscle action potential) of tibial nerves decreased in symptomatic recipients. The amyloid deposition was detected in 7 recipients (at age of 58.3 ± 9.8 years), and the mean duration from DLT to detection of amyloid deposits was 6.2 ± 3.5 years (1 to 12 years) in routine pathological examination. While we re-evaluated deposition of amyloid in the samples of 5 recipients with amyloid deposits, amyloid deposits were found on the samples of 2 recipients, previously judged as “negative deposits of amyloid”. In one recipient treated with oral intake of diflunisal from asymptomatic stage, amyloid deposits were first detected 12 years after DLT.

DISCUSSION & CONCLUSIONS

Clinical symptoms in DLT recipients seem to start unexpectedly earlier than in FAP patients. In addition, amyloid fibrils begin to accumulate from very early stage after DLT in some recipients and initial amyloid-lesions may have so far been overlooked in routine pathological examination. While it remains unclear from when oral intake of TTR-stabilizes should be begun, our results suggest that they should be started at least soon after identification of amyloid deposits, if possible from asymptomatic stage, since TTR-stabilizers may have a therapeutic effect to delay development of transmitted amyloidosis, as shown in one recipient with FAP (V30L) liver.

REFERENCES

The first pathological and biochemical identification of seed-lesions of transmitted transthyretin amyloidosis after domino liver transplantation

T Yoshinaga1, M Yazaki2, Y Sekijima1,2, F Kametani3, N Hachiya4, K Higuchi2, S Ikeda1,2

1Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan. 
2Institute for Biomedical Sciences, Shinshu University, Matsumoto, Japan. 
3Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan. 4Department of Neurophysiology, Tokyo Medical University, Tokyo, Japan. kiccho828@gmail.com

INTRODUCTION

The most serious issue in domino liver transplantation (DLT) using liver grafts from patients with transthyretin (TTR)-related familial amyloid polyneuropathy (FAP) is the development of iatrogenic transmitted amyloidosis (de novo amyloidosis) in DLT-recipients. However, little is known regarding the mechanisms of the initial stage of amyloid formation in these recipients. We detected initial lesions (possible seed-lesions) of this iatrogenic amyloidosis in two recipients following liver grafting from FAP patients. The deposited amyloid lesions were quite small, especially in recipient 1, we successfully isolated the amyloid fibrils using an advanced laser microdissection (ALMD) system which had specially been developed for isolation of intraneuronal inclusions [1].

MATERIAL & METHODS

The recipient 1 underwent DLT at age 65 from an FAP patient with a Val30Met TTR variant and the recipient 2, from an FAP patient with a Val30Leu TTR variant at age 32. The recipient 2 was started on diflunisal administration from 4 years after DLT because the FAP donor died one year after DLT due to severe progression of amyloid cardiomyopathy [2]. While either recipient had no clinical symptoms of FAP, quite small amyloid deposits were detected on the gastroduodenal mucosae only 14 months and 12 years after DLT in recipients 1 and 2, respectively. We isolated the tiny amyloid deposits from the gastroduodenal mucosae using an ALMD system and biochemically analyzed by tandem mass spectrometry (LC-MS/MS).

RESULTS

Biochemical analysis revealed that the amyloid was composed mostly of variant TTR, produced from the transplanted liver in both recipients. In follow-up study in recipient 1, wild-type TTR amyloid was detectable in the duodenal mucosa at 2 years after DLT.

DISCUSSION & CONCLUSIONS

This is the first study to successfully capture the pathological and biochemical features of initial-stage amyloid lesions in DLT recipients. So far, the shortest periods from DLT to identification of amyloid deposits have been reported as 3 years in the skin [3] and 4 years in the GI tract [4]. Our biochemical findings indicate that amyloid deposition can start by deposition of variant TTR from very early postoperative stage, followed by deposition of wild-type TTR, and blocking of amyloid seed formation from variant TTR may be a key to prevent or delay the development of DLT-associated amyloidosis.

REFERENCES

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Gastrointestinal symptoms in liver transplanted patients with hereditary transthyretin amyloidosis compared to healthy controls

Jonas Wixner¹, Therese Marberg¹, Intissar Anan¹ and Pontus Karling¹

¹Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

Background

Gastrointestinal (GI) complications are common in hereditary transthyretin amyloid (ATTR) amyloidosis, and previous studies indicate that the symptoms tend to increase after liver transplantation [1]. The aim of the present study was to perform a detailed evaluation of the frequency and severity of GI symptoms in liver transplanted patients with ATTR amyloidosis compared to that of healthy controls.

Materials and methods

The Gastrointestinal Symptoms Rating Scale (GSRS) is a validated self-assessment questionnaire for evaluating GI symptoms using a seven-point Likert scale. The scale contains 18 items, which can be grouped into seven symptom clusters. This questionnaire, together with a questionnaire on concomitant medications and diseases, was sent to 92 Swedish patients who had been transplanted for ATTR amyloidosis between 1990 and 2012. Healthy controls were randomly selected from the Swedish Betula study, a prospective study on memory, health and aging in a normal population.

Results

Seventy-seven patients and 299 age- and gender-matched controls were included in the study. All patients except two carried the TTR V30M mutation. GI symptoms were more frequent in patients than in controls, and the difference was highly significant (p <0.01) for five of the seven symptom clusters, however, no significant difference was found for gastroesophageal reflux disease (GERD) and dyspepsia. Abdominal pain, bloating and diarrhea were the most common symptoms among patients with a prevalence of 40-53%, whereas constipation and satiety were the symptom clusters with the highest proportion of patients reporting severe or very severe discomfort (6% and 3%, respectively). Patients reported higher total GSRS scores than controls (median score 26 vs. 8, p <0.01), and they also displayed higher symptom scores for six out of seven symptom clusters (p <0.01). GERD was the only symptom cluster for which no difference was found between the groups (p = 0.33). Logistic regression analysis showed no significant association between the total GSRS score, age, gender, age at onset or polypharmacy (five or more concomitant medications), however, there was a tendency towards higher symptom scores for polypharmacy (OR 2.20, CI 0.76-6.40).

Discussion and conclusions

Expectedly, patients who had been liver transplanted for ATTR amyloidosis showed a higher frequency and severity of GI symptoms compared to healthy controls. Gastroesophageal reflux and dyspepsia, however, did not appear to be a major problem for these patients. To better understand the mechanisms behind the patients’ GI symptoms after transplantation, future studies comparing liver transplanted ATTR amyloidosis patients to those transplanted for other diseases would certainly be of interest.

References

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Increasing Volume and Improved Survival in Solid Organ Transplantation for Amyloidosis. An Analysis of the UNOS database.

J Nativi-Nicolau,1,2,3 J Jaramillo1, J Fang1, R Al-Dulaimi4, T Kovacsovics1,2,3, J Abraham1,2,3, Mathew Maurer4, Josef Stehlik1,3

1University of Utah Health Science Center, Salt Lake City, Utah; 2 Huntsman Cancer Institute, Salt Lake City, Utah; 3 Utah Amyloidosis Program, Salt Lake City, Utah; 4 Columbia Presbyterian Medical Center, New York, NY

jose.nativi@hsc.utah.edu

Introduction: Amyloidosis is a rare disease that results from accumulation of inappropriately folded proteins causing organ failure and poor prognosis. In the last decade several options for therapy have emerged. We hypothesized that the trends and outcomes of solid organ transplantation (Tx) have increased and improved.

Material and Methods: We analyzed the UNOS database and included patients with diagnosis of amyloidosis who received a primary single organ (Kidney, Liver and Heart) or combined organs Tx (Heart-Kidney, Heart-Liver) between January 1987 and September 2014. We compared post-transplant outcomes in two separate eras: January 1987 to December 2004 (Era 1) and January 2005 to September 2014 (Era 2).

Results: From 1987 to 2014 a total of 1227 patients with amyloidosis were transplanted: kidney 591(48%), liver 249 (20%), heart 214(17%), heart-liver 50(4%) and heart-kidney 23(2%) (Figure). The five year survival for kidney tx improved from Era1 to Era2 from 66% to 82%, for liver tx from 54% to 70% and for heart tx from 54% to 69% (Figure). In a multivariate analysis, prominent risk factors for 1-year mortality after transplant for amyloidosis included recipient age for kidney (HR 1.06, p 0.01) and mechanical circulatory support (HR 5.42, p = 0.001) and body mass index (HR 0.9, p = 0.05) for heart transplant.

Discussion and Conclusions: More patients with amyloidosis are considered for solid organ transplantation. Outcomes continue to improve and are now approaching survival rates comparable to non-amyloid patients.
Clinical profile and outcomes of patients undergoing combined heart and liver transplantation with transthyretin amyloidosis: A report from the International Consortium on Cardiac Amyloid Transplantation (iCCAT)

KC Verkouw, M Vaduganathan, JR Stone, M Maurer, T De Marco, V Selby, J Estep, R Witteles, J Patel, M Hanna, M Zucker, D Baran, MJ Semigran

1 Massachusetts General Hosp, Boston, MA.  2 Columbia Univ Medical Ctr, New York, NY.  3 Univ of California San Francisco Medical Ctr, San Francisco, CA.  4 Houston Methodist Hosp, Houston, TX.  5 Stanford Univ Medical Ctr, Stanford, CA.  6 Cedars Sinai Hosp, Los Angeles, CA.  7 Cleveland Clinic, Cleveland, OH.  8 Newark Beth Israel Medical Ctr, Newark, NJ.

INTRODUCTION: Orthotopic heart transplant (OHT) is an accepted treatment option for transthyretin amyloidosis (ATTR) patients with end-stage heart failure. The incremental survival benefit of adjunctive orthotopic liver transplant (OLT) remains poorly defined.

MATERIAL & METHODS: We aim to describe the clinical profiles/prognoses of patients undergoing combined OHT/OLT compared with OHT alone. From January 1998 through May 2015, 42 of 48 ATTR patients being evaluated for OHT at 10 institutions underwent transplant and were included in the final analysis. Data were entered into an encrypted online database, Research Electronic Data Capture. The primary endpoint of post-OHT all-cause mortality was compared between 3 cohorts defined by transplant and mutation status using Kaplan-Meier survival analysis: OHT alone-wild type (WT) (n=12; Cohort A); OHT alone-ATTR mutation (n=21; Cohort B); and combined OHT/OLT-ATTR mutation (n=9; Cohort C).

RESULTS: Of the 21 variant ATTR subjects undergoing OHT alone the mutations were as follows: V122I (n=14), D38A (n=1), P44S (n=1), T59K (n=1), and unknown mutation (n=4). For the subset of 9 patients who underwent combined OHT/OLT (4 concurrently, 5 sequentially), the mutations were as follows: V122I (n=3), T60A (n=3), S23A (n=2), and S77Y (n=1). Baseline characteristics did not differ significantly across the 3 cohorts for age, LV ejection fraction, and other demographic, clinical, and hemodynamic characteristics. Post-OHT all-cause mortality was similar across cohorts. There was a trend toward shorter survival for cohort C when compared with cohort A (log-rank P=0.09; Figure 1).

DISCUSSION & CONCLUSIONS: This is one of the largest analyses of ATTR cardiac amyloidosis patients receiving heart and liver transplantation. ATTR patients receiving OHT/OLT versus OHT alone have similar median-term survival. The added survival benefit of liver transplantation to OHT in ATTR mutant patients should be investigated further.

Fig. 1: Survival of Cohorts from time of OHT - Log rank p-value compares the two variant groups (Cohorts B and C)
De novo familial amyloid polyneuropathy (FAP) in a FAP liver recipient

S Guttmann¹, C Röcken², M Schmidt¹, A Zibert¹, J Stypmann¹, M Schilling¹, HH Schmidt¹

¹Klinik für Transplantationsmedizin, Universitätsklinikum Münster, Germany. ²Institut für Pathologie, Universitätsklinikum Kiel, Germany. ³Department für Kardiologie und Angiologie, Universitätsklinikum Münster, Germany. ⁴Klinik für Allgemeine Neurologie, Universitätsklinikum Münster, Germany.

hepar@ukmuenster.de

Organ scarcity in the field of transplantation resulted into the idea of living related organ donation. Since familial amyloid polyneuropathy (FAP) usually results in a symptomatic phenotype within 3 or more decades, FAP liver transplant recipients were asked to donor their liver. One of the criteria was the older the age of the recipient the more safe this option may be. Recently, there is accumulating evidence, that FAP liver transplant recipients may develop de novo amyloidosis as early as about 8-10 years after transplantation. This accelerated presentation of FAP symptoms is of greatest concern in the policy the so-called domino liver transplantation concept. Trigger factors may include chronic inflammation such as in chronic viral hepatitis B/C, preexisting subclinical neurotoxicity such as in alcoholic induced liver failure and diabetes mellitus or the use of tacrolimus. Here we present a patient, who received a FAP TTRG47R domino liver after diagnosis of hepatocellular carcinoma and alpha 1-antitrypsin deficiency. Ten years after transplantation, the patient presents sensitivity disorders in distal extremities such as fingers and toes, neuropathic pain and intestinal abnormalities. To clarify these symptoms neurological examination was done. The diagnostic findings revealed a length dependent axonal senso-motoric polyneuropathy, which is likely during an early phase of amyloidosis. Due to blood pressure fluctuations and reduced physical resilience a cardiac involvement was suspected. Diagnosis by transthoracic echocardiography and heart muscle biopsy confirmed a concentric cardiac hypertrophy of the left ventricle, green bi-refringence of Congo red stained sections and immunohistochemical confirmation of tranthyretin deposits. Coprecipitation e.g. of alpha 1-antitrypsin was not detectable. The patient was immediately listed for liver retransplantation, but organ allocation regulations in Germany fail to offer an immediate liver allocation in this particular case. As bridging concept Tafamidis was administered. This case describes an early onset of FAP with cardiac involvement shortly after domino-transplantation and therefore highlights their risks and the need for further investigations.
Optimizing outcomes after heart transplantation in patients with cardiac amyloidosis - a single center analysis of 43 patients in 2 eras

Michael M. Kreusser1,2, Arnt V. Kristen1, Patrick Blum1, Ramon Tschierschke, Stefan O. Schönland3, Ute Hegenbart3, Arjang Ruhparwar4, Hugo A. Katus1,2, Philip W. Raake1

1Department of Cardiology, Angiology and Pneumology, University of Heidelberg, Germany
2DZHK (German Centre for Cardiovascular Research), partner site Heidelberg/Mannheim, Germany
3Department of Internal Medicine V, University of Heidelberg, Germany
4Department of Cardiac Surgery, University of Heidelberg, Germany

Introduction The prognosis of symptomatic cardiac amyloidosis - either of light chain (AL) or transthyretin (ATTR) type - is poor due to limited causative treatment options. Heart transplantation (HTX) might enable causative therapy and ultimately improve prognosis. However, previous reports suggested unfavorable survival after HTX in this population compared to HTX recipients with other indications as ischemic or dilative heart disease.

Methods and results Forty-three patients with cardiac amyloidosis (AL n= 29; ATTR n=14) underwent HTX at the University of Heidelberg Amyloidosis Centre between 2001 to 2015 and were analyzed retrospectively. In all patients with cardiac amyloidosis an exceptional high-urgency (HU) status was requested at Eurotransplant due to extremely poor prognosis. We analyzed the patients in two separate eras: 2001 to 2007 (era 1) and 2008 to 2015 (era 2). The time point separating these two eras was selected because it followed the initial publication of selection criteria for patients with AL amyloidosis undergoing HTX and autologous stem-cell transplantation (ASCT), and because it coincided with more restricted selection of patients as well as significant advances in chemotherapy of AL amyloidosis. Patients were subjected to continuous follow up after HTX in our outpatient clinic and a Kaplan-Meier survival analysis was performed. Both groups (era 1 and 2) were compared to all other patients, who received HTX because of other indications than amyloidosis and who were listed at Eurotransplant in HU status (n=174).

Patient characterization did not reveal significant differences in era 1 compared to era 2 except a lower number of organs affected by amyloidosis (1.8 ± 0.2 vs. 1.3 ± 0.1; p<0.05) in era 2 (2008-2015), reflecting a more restrictive patient selection in this era. However, while patients in era 1 (2001 to 2007) showed impaired survival after HTX compared to the control HU group. Interestingly, in era 2 the survival of amyloidosis patients measured up to the survival of the other HU HTX patients and was significantly improved compared to era 1 amyloidosis HTX patients.

Conclusions HTX in advanced cardiac amyloidosis - either of AL or ATTR type - is a promising approach to allow causative treatment and finally improve poor survival of cardiac amyloidosis patients. Our data demonstrates that HU HTX - for AL combined with subsequent stem cell therapy - offers a successful treatment option to improve the poor outcome of this population. Furthermore, the data demonstrates that outcome of patients improved in era 2 (2008-2015) compared to era 1 (2001-2007). This era effect appears to be related to patient selection restricted to patients with sole cardiac involvement. Our data demonstrates that HTX in advanced cardiac amyloidosis should be restricted to highly selected patients in specialized centres.
Liver transplantation is a potential treatment option for systemic light chain amyloidosis patients with dominant hepatic involvement: A case report and analytical review of the literature

A Ueno¹, N Katoh¹, T Yoshinaga¹, O Aramaki², M Makuuchi², Y Sekijima¹, and S-I Ikeda¹

¹ Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan. ² Department of Hepato-Biliary-Pancreatic-Transplantation Surgery, Japanese Red Cross Medical Center, Shibuya-ku, Japan.

uenakhr@gmail.com

INTRODUCTION

Systemic light chain (AL) amyloidosis is caused by abnormal plasma cell clones in the bone marrow. Better prognosis can be expected if standard chemotherapy achieves sufficient reduction of amyloid precursor protein. However, advanced organ involvement, typically cardiac involvement, sometimes limits the indication of chemotherapy and results in poor outcome. Clinical problems due to hepatic involvement also sometimes prevent patients from receiving effective chemotherapy. Therefore, liver transplantation (LT) has ever been applied in some patients with dominant hepatic impairment as a possible therapeutic option. However, its outcome has not been necessarily favorable. In order to provide a better outcome, the possible prognostic factors of this treatment strategy were investigated.

MATERIAL & METHODS

We report a systemic AL amyloidosis patient who presented with dominant hepatic involvement and was therefore received LT. We also reviewed and analyzed previous literature in which LT was performed to the patients with advanced hepatic AL amyloidosis.

RESULTS

Case report: A 58-year-old man with hepatic AL amyloidosis was referred to our department for further examination and treatment. However, he was considered to be ineligible for standard chemotherapeutic approaches due to progressed hepatic failure with elevated total bilirubin (T-bil, 1.93 mg/dL) and alkaline phosphatase (ALP, 1996 IU/L). His cardiac and renal involvements were trivial. After restoring his liver function by preceding living-donor partial LT, he provided good hematological response and long survival for 32 months so far under standard chemotherapy.

Review of the literature: Besides our case, there have been 15 cases of hepatic AL amyloidosis treated by LT. Among them, only eight patients (50%) survived for more than 12 months. The patients were divided into two groups depending on the outcome at 12 months (survivors and nonsurvivors) and their clinical backgrounds were compared. The number of patients with cardiac involvement in the group with poor prognosis (4 of 8) was double that in the group with better prognosis (2 of 8) but there was no statistical significance. Other organ involvements were not significant. Several laboratory parameters including T-bil, ALP, free light chain, and cardiac biomarkers were investigated but statistical analysis was not possible due to lack of data. The living-donor approach did not affect the outcome. Pre-LT chemotherapy and perioperative complication(s) were not significant predictors. Post-LT chemotherapy was found to be an only significant prognostic factor in the present study ($P = 0.020$).

DISCUSSION & CONCLUSIONS

Considering the essentially progressive nature of systemic AL amyloidosis, it seems reasonable that post-LT chemotherapy, which may halt or decelerate disease progression, would determine the prognosis. Hence, the most important point to consider for clinicians treating AL patients awaiting LT is to maintain their eligibility for curative chemotherapy. The eligibility criteria of chemotherapy vary among institutions and chemotherapy regimens, but preserved cardiac function are universally required. Therefore, careful attention to cardiac involvement is required when treating patients with hepatic amyloidosis at the time of diagnosis, during the perioperative period of LT, and when initiating chemotherapy.
Clinicopathological and biochemical findings of late-onset hereditary transthyretin amyloidosis 16 years after liver transplantation: an autopsy case study

M Mizukami¹, M Ueda¹, M Tasaki¹², Y Misumi¹, T Masuda¹, S Matsumoto¹, T Yamashita¹, Y Ando¹

¹ Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan  ² Department of Morphological and Physiological Sciences, Graduate School of Health Sciences, Kumamoto University, Kumamoto, Japan

bisco4917@yahoo.co.jp

INTRODUCTION:
Hereditary transthyretin (TTR) amyloidosis patients have undergone liver transplantation (LT) as treatment for the disease. LT reportedly prolonged survival of early-onset hereditary ATTR amyloidosis patients with the ATTR Val30Met mutation, when they were selected in the strict criteria. However, pathological changes in late-onset hereditary ATTR amyloidosis long time after LT were still not well understood. In this study, we investigated pathological changes in an autopsied late-onset hereditary ATTR amyloidosis case 16 years after LT.

METHODS:
We investigated histopathological and biochemical findings in various tissue sites using tissue specimens obtained from an autopsied late-onset hereditary ATTR amyloidosis case 16 years after LT.

RESULTS:
A 73 year-old-man suffering from hereditary ATTR Val30Met amyloidosis died 21 years after onset of the disease and 16 years after LT. Autopsy study revealed that he had severe amyloid deposits especially in the heart, tongue, and ligaments. Mass spectrometric analyses revealed that amyloid deposits in the heart, tongue, and ligaments derived mostly from wild type TTR, which was secreted from the transplanted liver graft. We also analyzed fragmentation of TTR using tissue specimens. The fragmentation was found in the heart, tongue, and ligaments, but not in other tissue sites.

CONCLUSION:
Pathological and biochemical changes may differ among tissue sites in hereditary TTR amyloidosis after LT.
Long-term effects of liver transplantation on small-fiber dysfunction in Japanese transthyretin (TTR)-related familial amyloidotic polyneuropathy (FAP) ATTR V30M

K Obayashi¹, M Ueda², T Yamashita², M Tasaki¹, A Izaki¹, Y Yanagisawa¹, T Masuda², Y Misumi⁵, Y Ando⁵

¹Department of Morphological and Physiological Sciences, Graduate School of Health Sciences, Kumamoto University, Kumamoto, Japan. ²Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan.

INTRODUCTION

In 1995, Ando et al. reported that several autonomic disturbances improved after liver transplantation in a Japanese FAP ATTR V30M patient (Lancet, 1995). On the other hand, Wiklund et al. reported that cardiac autonomic function does not improve after liver transplantation in Swedish transthyretin (TTR)-related familial amyloidotic polyneuropathy (FAP) patients (Auton Neurosci, 2010). However, the outcome of autonomic function in various organ after liver transplantation in TTR-FAP is still controversial. The aim of this study was to investigate the long-term effects of liver transplantation on autonomic dysfunction in Japanese FAP ATTR V30M patients.

MATERIALS & METHODS

Thirty-six Japanese transplanted FAP ATTR V30M patients (15 male and 21 female) were assessed. We compared with the various autonomic data between before and after liver transplantation in each patient.

RESULT

All the results of this study are shown in Table 1.

<table>
<thead>
<tr>
<th>Improvement rate of autonomic dysfunction after liver transplantation</th>
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<tr>
<td>1. Cardiac R-R interval (CVR-R, LF/HF ratio)</td>
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<td>2. Pain threshold on foot</td>
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<td>3. Urinary incontinence</td>
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<td>4. Finding from study of ¹²³I-MIBG myocardial</td>
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<td>5. Temperature threshold on foot</td>
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<td>6. Dry eye</td>
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<td>7. Dry mouth</td>
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<td>8. Dyshidrosis</td>
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<td>9. Light reflex</td>
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<td>10. Electile dysfunction</td>
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<td>• Orthostatic hypotension</td>
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<td>• Degree of diarrhea/constipation</td>
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DISCUSSION & CONCLUSIONS

Autonomic symptoms definitely improved in some liver-transplanted patients especially in the patients who showed mild autonomic dysfunction before the operation. Some preoperative data, such as CVR-R and/or LF/HF ratio in cardiac R-R interval, pain threshold on foot, level of urinary incontinence, and finding from study of ¹²³I-MIBG myocardial scintigraphy, may be useful predictive markers of prognosis for autonomic dysfunction after liver transplantation. On the other hand, the level of orthostatic hypotension and degree of diarrhea/constipation may not be useful markers because of their big fluctuation.
Iatrogenic systemic transthyretin amyloid deposits in a case with domino liver transplantation: an autopsy case study

Y Tsuda, Y Misumi, M Ueda, M Tasaki, G Huang, T Masuda, G Suenaga, Y Inoue, Y Kinoshita, K Obayashi, T Yamashita, Y Ando

INTRODUCTION
Domino liver transplantation (DLT) using liver grafts from patients with hereditary transthyretin (TTR) amyloidosis has been performed because of a severe liver graft shortage. Some of these DLT recipients develop iatrogenic TTR amyloidosis from 3 to 9 years after DLT. The objective of this study was to elucidate clinicopathological and biochemical characteristics of an autopsy case with iatrogenic systemic TTR amyloid deposits after DLT.

METHODS
We investigated clinicopathological and biochemical characteristics of a 61-years-old female autopsy case with iatrogenic systemic TTR amyloid deposits who died 8 years after DLT.

RESULTS
TTR amyloid deposits were found in various kinds of tissue sites such as heart, gastrointestinal tract, tongue, kidneys, thyroid gland, and peripheral nerves. Mass spectrometric analyses revealed that those amyloid deposits were derived mostly from full-length mutated TTR.

DISCUSSION & CONCLUSIONS
It has been generally reported that patients with late-onset hereditary TTR amyloidosis have fibrils with fragmented TTR and higher wild-type TTR proportion. However, this case showed fibrils with mostly full-length and higher mutated TTR proportion. These results may indicate that iatrogenic TTR amyloid formation in the DLT recipients is different from that in usual patients with hereditary TTR amyloidosis.
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Occurrence factors and clinical picture of iatrogenic transthyretin amyloidosis after domino liver transplantation

Y Misumi¹, T Oshima¹, M Ueda¹, T Yamashita¹, M Tasaki¹², T Masuda¹, K Obayashi², Y Ando¹

¹Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan,
²Department of Morphological and Physiological Sciences, Graduate School of Health Sciences, Kumamoto University, Kumamoto, Japan

misumiyohei@hotmail.co.jp

INTRODUCTION

Domino liver transplantation (DLT) with liver grafts from patients with hereditary transthyretin (TTR) amyloidosis has been performed for patients with fatal hepatic disorders. Since 2004, several cases with symptomatic and asymptomatic amyloid deposition after DLT was reported, however, the precise pathogenesis and clinical course of iatrogenic TTR amyloidosis remains unclear.

METHODS

We analysed the occurrence factors and clinical features of iatrogenic TTR amyloidosis in consecutive 23 domino liver recipients who received the liver from hereditary TTR amyloidosis.

RESULTS

The mean times from DLT to amyloid first appearance and symptom onset in DLT recipients were 8.2 years and 9.9 years, respectively. Kaplan-Meier analysis and quantification of the amyloid deposition indicated that older recipients tended to develop amyloid deposition earlier than younger recipients. Most patients with iatrogenic TTR amyloidosis developed with sensory disturbance in the lower extremities as an initial symptom, and showed minimal autonomic dysfunction. These clinical features were distinct from those of typical hereditary TTR amyloidosis with ATTR Val30Met mutation.

DISCUSSION & CONCLUSIONS

Our results indicate that the clinical picture of iatrogenic TTR amyloidosis in the DLT recipients is different from that in FAP patients. Iatrogenic amyloidosis will likely increase, careful follow-up and new treatments are required.

REFERENCE

AA Amyloidosis: An evaluation of epidemiology and prevalence in the US and EU5 countries

W Andrews¹, D Garceau¹, T Sablinski¹, P Zhang², T Waldman²

¹Auven Therapeutics, St. Thomas, USVI, ²Navigant Consulting, Inc., New York, USA

INTRODUCTION:
AA amyloidosis is a rare, systemic form of amyloidosis characterized by abnormal deposition of amyloid A proteins, which can be deposited in a variety of organs but often impacts the kidneys. The disease is secondary to several chronic inflammatory conditions and treatment of the inflammation can reduce manifestations of AA Amyloidosis in some patients¹. In many patients, adequate suppression of inflammation is not possible and, as well, there is a significant delay in diagnosis. As a result, AA Amyloidosis is associated with significant morbidity and mortality¹,². As with most rare diseases, understanding the epidemiology and prevalence of the disease is difficult, but is critical to improving the ability to diagnose the disease in a timely manner such that optimal treatment can be provided to patients². As there is no clear source that defines the prevalence of AA Amyloidosis, we sought to evaluate prevalence in the US and EU5 through multiple methodologies.

MATERIALS & METHODS:
AA amyloidosis prevalence was derived through a blend of the most appropriate methodologies applicable to each region based on a robust literature review, discussions with KOLs, and a quantitative survey. The methodologies used included: 1) Evaluation of AA Amyloidosis diagnosis rate via renal biopsy with extrapolation to that regional population based on overall rate of renal biopsy, 2) Assessment of multiple regional centers of excellence with region-specific patient registries and/or databases, with extrapolation to those regional populations, and 3) A qualitative survey of 200 physicians (150 U.S. and 50 EU) including Nephrologists, Rheumatologists, and Gastroenterologists to assess numbers and rates of AA Amyloidosis patient diagnoses. These methods were used to derive an estimation of prevalence by geography.

RESULTS:
Estimates suggest that there are approximately 15,300 AA Amyloidosis patients currently under the care of a physician in the U.S. and EU5, comprising ~9,100 patients in the U.S. and ~6,200 patients in the EU5. In regards to the patient pathway to diagnosis in the U.S. and EU5, the diagnosis is typically queried by either a Generalist or the specialty physician treating the underlying inflammatory disorder. The patient is then referred to a Nephrologist for confirmation of the diagnosis, which is most often done by renal biopsy. A difference between care in the U.S. and the EU5 is that care is more centralized in the EU5, with each country having its own AA Amyloidosis referral center. In Italy, Spain, and the UK, over 50% of patients are treated in these centers of excellence. In France and Germany, physicians are less likely to refer patients to their AA Amyloidosis centers.

DISCUSSION & CONCLUSIONS:
Understanding the prevalence of a rare disease is typically very difficult due in large part to the small numbers of patients with the disease spread out across the globe. Other reasons are low awareness of the disease and variations between countries and regions in regards to: clinical practice, the patient pathway from generalists to specialists, the availability and affordability of specialized diagnostic testing, and the existence of centers of excellence. In addition, global registries are not in place for most rare diseases. In this evaluation, five different methodologies were evaluated for use in the estimation of prevalence for AA Amyloidosis and three of these five were chosen to be robust approaches for regionally specific estimations and subsequent blending of these methodologies for a broader prevalence estimation. This research suggests an AA Amyloidosis prevalence of approximately 15,300 patients in the US and EU5. Bringing together centers of excellence and thought leaders across multiple regions to develop a global patient registry would help to further define the epidemiology and prevalence of this rare but devastating disease.

REFERENCES:
Amyloid A (AA) amyloidosis in CByB6/F1 mice: a model to study gastrointestinal amyloidosis

AY Gahane, AK Thakur

Department of Biological Sciences and Bioengineering, Indian Institute of Technology Kanpur, (UP), India-208016.
akthakur@iitk.ac.in

INTRODUCTION: AA amyloidosis is characterized by extracellular deposition of amyloid fibers of abnormally degraded serum amyloid A (SAA) protein in various organs, resulting in cellular damage and organ dysfunction [1]. Gastrointestinal (GI) tract is one of the most common site (apart from spleen, liver and kidney) affected in AA and other forms of systemic amyloidosis [2]. Previously studied animal models of AA amyloidosis (BALB/c, C57BL/6, CBA, NMRI mice models) showed spleen and liver as dominant organ for deposition of AA amyloid fibrils [3]. Here we show that gastrointestinal specific AA amyloid deposition occurs in CByB6/F1 mouse model.

MATERIAL & METHODS: Male and female CByB6/F1 hybrid mice, 8-12 weeks of age, were kept in a 12:12 h light-dark cycle before and during the entire experiments. AA amyloidosis was induced by administration of a single dose of amyloid enhancing factor (AEF) (100 ug; i.v.) in combination with 0.5 ml of 2% AgNO₃ (s.c.), periodically over 17 days. Apart from this, inflammatory agents like lipopolysaccharides (LPS) in combination with AEF and azocasein alone have been used. GI amyloidosis was analyzed by Congo red staining of tissues, derived from stomach and intestinal mucosa. The blood samples were collected at different time intervals through retro-orbital route in order to determine the variation in plasma SAA concentration during development of GI amyloidosis using SAA mouse ELISA kit.

RESULTS: Gastrointestinal amyloidosis was seen in test group mice within 10 days of AEF and AgNO₃ administration. The Congo red stained, large and small amyloid deposits, showing apple-green birefringence under polarized light, were present within stomach and intestinal walls. Deposits were also present in the fecal matter. Similar results were obtained in 3 days with LPS (in combination with AEF) and in 22 days of azocasein administration. Splenomegaly and hepatomegaly was seen in all animals, representing severe inflammation. As consistent with previous studies, AgNO₃ treatment results in 67 fold (1298 ug/ml on day 6), while LPS treatment showed 85 fold (1710 ug/ml on day 3) increase in plasma SAA level to that of initial concentrations. Very high SAA concentration might be the reason for early development of GI amyloidosis in LPS treated mice. However, no amyloids were detected in spleen, liver or kidney in either of the treatment.

DISCUSSION & CONCLUSIONS: Using CByB6/F1 mice model, we showed GI specific AA amyloid deposition. The exact mechanism of the presence of amyloid accumulation in GI and not in spleen or liver needs further investigation. We speculate the role of heterogeneous population of peritoneal macrophages in degradation of intestinal derived and infiltrated plasma SAA in gastrointestinal amyloidosis development. The model could be useful in finding new insights of pathological mechanism and histo-pathological changes in gastrointestinal amyloidosis.

REFERENCES:

AA amyloidosis in father and daughter as complication of PAPA syndrome (PSTPIP1 E250K mutation)

H.L.A. Nienhuis¹, A.J van Essen², J. Bijzet¹, J.S.F. Sanders³, R.W.J. van Rheenen⁴, and B.P.C. Hazenberg¹

¹Departments of Rheumatology & Clinical Immunology, ²Medical Genetics, ³Nephrology, and ⁴Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

h.l.a.nienhuis@umcg.nl

INTRODUCTION: AA amyloidosis is the result of some chronic inflammatory process. In most cases the underlying disease can be found, but some case remain idiopathic. Especially in case of familial occurrence of AA amyloidosis, a common hereditary cause is suspected to be present.

CASE HISTORIES:

A man, born in 1956, suffered from acute rheumatic fever when he was 8 years old, furunculosis and aseptic osteomyelitis (13 years), followed by splenomegaly and granulocytopenia when he was 14 years old. At the age of 22 he was analyzed for extensive acne and at the age of 32 he developed pyoderma gangrenosum. A diagnosis of SAPHO was made. At the age of 37 he suffered from arthritis of MTP2 of the right foot. At 40 he was analyzed because of his chronic idiopathic neutropenia and he received G-CSF with correction of the neutropenia, but he did not tolerate it at all. Three months later he developed diarrhea, weight loss and progressive loss of renal function. A rectum biopsy showed AA amyloid to be present. SAP scintigraphy showed increased uptake in spleen, kidneys and adrenal glands. The renal failure progressed and he died suddenly at the age of 41. DNA was stored.

His daughter was born in 1986. She had a congenital granulocytopenia and had a sterile pyogenic arthritis of the left knee when she was 12 years old and thereafter this recurred twice in the right knee. She did not tolerate G-CSF treatment at all. At the age of 16 she turned out to have hepatomegaly (14 cm) and splenomegaly (14.5 cm). She did not suffer from prominent acne or pyoderma like her father. In 2008, when she was 20 years old, she developed a nephrotic syndrome and the kidney biopsy showed AA amyloidosis. SAP scintigraphy showed increased uptake in an enlarged spleen, kidneys and adrenal glands. She was treated with colchicine with little results, she developed renal failure in 2013 and started with hemodialysis. Extensive genetic analysis for auto-inflammatory syndromes and congenital granulocytopenia had not been successful until 2016 when it turned out that she had the E250K mutation of PSTPIP1 (c.748G>A) and this mutation was also detected in retrieved DNA of her father.

The PSTPIP1 mutations are related to PAPA syndrome, an acronym of pyogenic arthritis, pyoderma gangrenosum and acne syndrome, a disease belonging to the spectrum of auto-inflammatory diseases (1-3).

DISCUSSION & CONCLUSIONS:

This is the first report of AA amyloidosis in both father and daughter who both also have PAPA syndrome as underlying inflammatory cause. The daughter does not display all phenotypic features of the syndrome (yet). The common clinical presentation of father and daughter with granulocytopenia and splenomegaly is striking. PAPA syndrome therefore may be a cause of idiopathic chronic granulocytopenia, of splenomegaly, and of AA amyloidosis.

REFERENCES:


Obesity as a possible susceptibility factor for AA amyloidosis

T Youngstein, T Lane, J Pinney, J Gilbertson, T Rezk, C Quarta, A Wechalekar, S Mahmood, S Sachchithanantham, JD Gillmore, PN Hawkins, HJ Lachmann.

National Amyloidosis Centre, Division of Medicine, Royal Free Campus, University College London, UK
t.youngstein@ucl.ac.uk

Introduction: Systemic AA Amyloidosis is a serious consequence of chronic inflammation leading to renal failure and death (1). The UK National Amyloidosis Centre (NAC) has accrued 655 cases since 1990. Over this period the diagnosis rate has remained constant, with a median of 24 diagnoses per annum (IQR 19-31). However, their aetiology has changed dramatically. From 1990-1997 the most prevalent underlying diagnoses were rheumatoid Arthritis (RA), juvenile idiopathic arthritis (JIA) and chronic sepsis. In contrast, between 2007-2016 the most common underlying diagnosis was “unknown”; no cause identified despite extensive investigations; RA is the second most common and injected drug abuse the third. There has been a 98% reduction in amyloidosis secondary to JIA, and a 30% reduction in cases due to RA, undoubtedly reflecting therapeutic advances with combined immunosuppressive and biologic therapies. Over the period there has been a 600% increase in cases of AA Amyloidosis of unknown origin. All individuals have been extensively investigated for known causes of inflammation or infection, including genetic sequencing for heritable periodic fever syndromes, CT and FDG-PET CT imaging. We sought to formally assess the role that obesity may play in these cases.

Materials & Methods: Baseline demographics including height, weight, underlying diagnosis, treatment and outcomes were recorded in all cases of AA amyloidosis. Body mass index (BMI) was used as an indirect marker of body fat (2), calculated using the formula: weight (kg)/[height (m)]². Reference ranges: BMI < 18.5 underweight, 18.5-24.9 normal, 25.0-29.9 overweight, >30 obese. Data was analysed using an unpaired t test (GraphPad Prism 6.0).

Results: 97 individuals with AA Amyloidosis of unknown origin were identified. 50% were male. Height and weight data was available in 73 (76%). A cohort of 104 cases of AA Amyloidosis, 33% male, with diagnoses of RA, seronegative arthritis, ankylosing spondylitis and systemic autoinflammatory disease was used for comparison. Mean age in the unknown cohort was 59.6 years and 57.3 years in controls (p=0.41). In the unknown cohort 60% were overweight, 39% obese. In the control group 43% were overweight, 27% obese. Mean BMI for the unknown cohort was 27.2 vs 23.6 in controls (p< 0.0001) (Figure 1).

Discussion & Conclusions: BMI is significantly higher in patients with AA Amyloidosis of unknown origin (p< 0.0001), suggesting that adipocyte production of pro-inflammatory cytokines may contribute to overproduction of serum amyloid A protein (SAA) in the 60% of the cohort who were overweight. Currently the UK population mean BMI is 27.4 in men and 26.9 in women, with 41% of men and 33% of women classified as overweight, and 26% and 24% as obese (3), raising the possibility that AA Amyloidosis might in future become more common among patients with less intense inflammatory disorders.

References:
The role of lipid membrane in development of AA amyloidosis

A Vahda shariat panahi1, P Hultman2, GT Westermark3, K Lundmark1

1Department of Clinical Pathology and Clinical Genetics, and Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden. 2Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden. 3Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden. aida.vahdat@liu.se

INTRODUCTION: AA amyloidosis results from aggregation and deposition of protein AA, the degradation product of acute phase reactant, serum amyloid A (SAA). In experimental mouse model, AA amyloidosis is induced by long-standing inflammatory stimulation. In vitro studies showed that lipid membranes accelerate amyloid fibril formation (1).

MATERIAL & METHODS: Multilayered cholesterol/phosphatidylcholine liposomes containing PBS (PBS-L) and two different preparations of fluorescent liposomes (PBS-FL) were used. To study the role of lipid membranes in amyloidogenesis in vivo groups of NMRI mice were injected with PBS-L i.v. followed by injections of AgNO3, s.c. Injections of AgNO3 were repeated after 7 and 14 days. Control mice were injected with PBS and AgNO3 as described or PBS-L i.v. Mice were sacrificed after 4, 10 or 16 days. Amyloid load was determined in spleen sections stained with Congo red (0 to 4+). In order to explore the fate of the liposomes groups of mice were injected with PBS-FL i.v. and sacrificed after 30 min to 4 days. The amount of PBS-FL in blood, spleen and liver was determined using Image J. To investigate intracellular localization of liposomes, Monocyte macrophages Cell line (J774A.1) was incubated with PBS-FL for 2, 6 and 8 hours, Lysotracker Red was applied to visualize lysosomes.

RESULTS: Amyloid deposits (1+-4+) developed in 4 of 6 mice 10 days after induction with PBS-L and AgNO3 and after 16 days all 7 mice had amyloidosis (1+-3+). No amyloid was detected in control groups. Intravenously injected PBS-FL were cleared from the circulation within one hour, uptake by both spleen and liver reached a peak within 3 hours and almost all liposomes were cleared from spleen and liver after 4 days. In vitro studies demonstrated that PBS-FL co-localized with lysosomes after being phagocytosed by macrophages.

DISCUSSION & CONCLUSIONS: Macrophages can endocytose SAA and intracellularly transform SAA into amyloid in vitro (2) and are crucial for amyloid formation in vivo (3). Sequestration of SAA in the lysosomes of monocytic cells from amyloidotic mice implicates the role of lysosomes in amyloid formation (4). Our results clearly show that liposomes accelerate amyloid development in inflamed mice. Phagocytosed liposomes accumulate in lysosomes and our hypothesis is that this accumulation of lipid membranes creates favorable conditions for formation of a nidus for further amyloid deposition.

Fig. 1: The amount of PBS-FL in blood, spleen and liver and the average of Congo red (CR) grading of amyloid in spleen in mice injected with PBS-L and AgNO3.

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Systemic AA amyloidosis associated with a malignant carotid paraganglioma

Simon Gibbs1,2, Louis Huang1 and Robert MacGinley1

1 - The Victorian and Tasmanian Amyloidosis Service, Eastern Health Monash University Clinical School, Melbourne; 2 - The Australian Amyloidosis Network

simon.gibbs@monash.edu

Introduction
Paragangliomas are neoplasms of the paraganglia within the paravertebral sympathetic and parasympathetic chains. The carotid body is a common site and these can be extremely slow growing. There are only a few cases worldwide of paragangliomas causing systemic AA amyloidosis (AA). We present a new case, with remarkable clinical improvement after peritoneal dialysis (PD).

Results
A 59 year old man presented with nephrotic syndrome, stage V CKD, and hypoalbuminaemia (14 g/L). There was a history of 8kgs weight loss over 12 months, chronic diarrhoea, reduced left ventricular function and symptomatic postural hypotension. Renal biopsy confirmed AA. Our patient had a past history of a 30 year history of an inoperable right carotid paraganglioma with L3/L4 vertebral metastases. He was awaiting a novel radio-oncological treatment, Lu-TATE, for these tumours at time of the diagnosis of AA.

Our patient was refused initially for PD by his local hospital, citing poor prognosis from AA; however, after discussion with our nephrologists and oncologists, PD was commenced at the Victorian and Tasmanian Amyloidosis Service. Various attempts were made to find the optimal clearance regimen. Dextrose from PD fluid was found to provide additional calories, while absorption of PD fluid helped raise blood pressure. Oral nutritional supplement was commenced to balance the catabolic state, as well as an antidepressant. Lu-TATE was commenced to treat the paragangliomas.

18 months after presentation, the patient had settled on PD, was emotionally stable, physically active, with no hypotension and free of peripheral oedema. Follow-up PET scanning revealed a decrease in the bulk of his paragangliomas.

Discussion and Conclusions

Carotid paragangliomas are an exceedingly rare cause of AA. New treatments for these tumours are now available that hopefully slow the progression to AA.

This case reinforces the role of PD, even in advanced cases of AA. Treatment with intensive supportive care and PD led to a marked improvement in patient symptoms and quality of life and allowed our patient to undergo Lu-TATE treatment for his paragangliomas, resulting in a reduction in his tumour load, further symptom relief and no doubt, improvement in overall survival.
Heparin interactions with apolipoprotein A1 and serum amyloid A in inflammation-associated high density lipoprotein

A Digre1, J Nan2, M Frank3, JP Li1

1 Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden. 2MAX IV Laboratory, Lund University, Lund, Sweden. 3Biognos AB, Gothenburg, Sweden.

andreas.digre@imbim.uu.se

INTRODUCTION: Apolipoprotein A1 (apoA1) is the main protein component responsible for transportation of cholesterol on high-density lipoprotein (HDL). Serum amyloid A (SAA) is an acute phase protein associated with HDL. Apart from their physiological functions, both apoA1 and SAA have been identified as ‘amyloidogenic peptides’ linked to heparin analog heparan sulfate. Our recent study has found that incubation of acute phase HDL (HDL-SAA) with heparin leads to deformation of the lipoprotein particles, and the dissociation of the HDL complex. As this dissociation is dependent on the molecular size of heparin, we proposed a model where both apoA1 and SAA are involved in binding to heparin.

MATERIAL & METHODS: HDL from normal mice and HDL-SAA from inflamed mice were isolated from plasma by sequential density flotation. The samples were incubated with heparin, separated by centrifugation and analyzed using SDS-PAGE gels and Western blotting. Relative quantification was done by Image Lab analysis software (Bio-Rad). The isolated HDL and HDL-SAA were also chemically crosslinked and analyzed with mass spectrometry (MS) to reveal possible crosslinks between apoA1 and SAA proteins.

RESULTS: Heparin interacts with both apoA1 and SAA in HDL isolated from plasma of inflamed mice. The reaction is rapid, forming complex aggregates composed of heparin, apoA1 and SAA as revealed by gel electrophoresis. This interaction is dependent on the size and concentration of added heparin. Mass spectrometry analysis of peptides derived from chemically crosslinked HDL-SAA particles detected multiple crosslinks between apoA1 and SAA, indicating close proximity (within 25 Å) of these two proteins on the HDL surface.

DISCUSSION & CONCLUSIONS: Our data provides experimental evidence for the molecular interaction of apoA1 and SAA with heparin and reveals the close proximity of apoA1 and SAA in the HDL-SAA particle. Though both apoA1 and SAA interact with heparin, they also appear to have distinctive individual features in this interaction.

REFERENCES:


Fig. 1: Effect of heparin size in dissociation of SAA and apoA1. HDL-SAA was incubated with heparin or heparin-derived oligomers at room temperature for 1 h. The resulting supernatant and pellet were analyzed on a gradient SDS-PAGE pre-cast gel (4-20%), followed by staining with Coomassie blue.
INTRODUCTION: Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory disease characterized by recurrent, self-limiting attacks of fever, and serositis. Renal amyloidosis is a serious complication of FMF that determines the prognosis. Patients with AA-Amyloidosis can be treated with Interleukin-1 (IL-1)-inhibiting drugs. We report our single center experience in adult patients with colchicine-resistant FMF-related amyloidosis who were treated with colchicine and anakinra.

MATERIAL & METHODS: Demographic data, clinical and laboratory parameters, MEFV mutations, patient reported outcomes and physician global health were extracted from our GARROD (German Anti-IL1beta medication RegistRy in Orphan Diseases) registry.

RESULTS: Within our cohort of 205 adult patients with FMF, we identified 36 patients (17.6%) with FMF-related amyloidosis. All patients received colchicine treatment. A subgroup of these patients continued to have colchicine-resistant FMF attacks and elevated acute phase reactants in the serum. Nine patients (2 female and 7 male) were treated with a combination of colchicine (1.5 +/- 0.65 mg per day) and Anakinra. All patients were of turkish-armenian ancestry. Homozygosity for high penetrance mutation was present in 7 patients, heterozygosity in 2 patients. Three patients (33%) presented with nephrotic syndrome, 3 patients (33%) with chronic kidney disease (CKD), one with end-stage renal disease (ESRD), and 2 patients were 5 and 7 years after renal transplantation.

Before initiation of anakinra the 24h urine protein excretion (mean +/-SD) was 5.0g +/-3.5g /24h. The proteinuria decreased with anakinra to 0.4g +/-1g /24h in the last visit after 24 +/- 18 months. The creatinine serum levels of all patients with CKD decreased or remained stable.

The patient reported health (VAS 70 +/- 21mm) and the physician reported global health (VAS 80 +/- 16mm) both improved significantly (VAS 10 +/- 20mm, VAS 7.5 +/- 20mm) (p 0.001, p 0.0009) after 6 months of treatment.

DISCUSSION & CONCLUSIONS: Anakinra is well tolerated and effective in patients with FMF-related amyloidosis.
First report of AA amyloidosis caused by low level somatic mosaicism in the NLRP3 gene in two patients with late onset cryopyrin-associated periodic syndrome (CAPS)

Dorota M Rowczenio1, Sónia Melo Gomes3, Juan I Aróstegui 2, Ebun Omoyinmi 3, Despina Eleftheriou1, Nigel Klein 1, Paul Brogan3, Helen J Lachmann 1 and Philip N Hawkins1

1 National Amyloidosis Centre, UCL, London, UK; 2 Hospital Clinic-IDIBAPS, Barcelona, Spain; 3 Institute of Child Health, UCL, London, UK.

d_rowczenio@ucl.ac.uk

Introduction: Cryopyrin-associated periodic syndrome (CAPS) is an autoinflammatory disease (AID) associated with mutations in the NLRP3 gene that cause overproduction of IL-1β. CAPS usually presents at birth and is usually inherited in a dominant heterozygous fashion, although de novo mutations occur. Advances in next-generation sequencing technologies have lately confirmed that some ‘mutation negative’ CAPS patients are mosaic for NLRP3 gene mutations. CAPS is characterised by recurrent episodes of fever, urticarial rash, arthralgia, myalgia, aseptic meningitis and inflammatory eye manifestations and can be triggered by exposure to cool or damp environment. The disease is usually accompanied by a striking acute phase response, and patients are therefore at high risk of developing AA amyloidosis.

We report here two patients who presented with AA amyloidosis, and were subsequently diagnosed with unusually late onset of CAPS caused by mosaic mutations in the NLRP3 gene.

Methods: Two unrelated patients: a 63- year old male (patient 1) and a 66- year old female (patient 2) presented with heavy proteinuria and were diagnosed with AA amyloidosis on a renal biopsy. To determine the aetiology of their disease they underwent detailed clinical and laboratory investigations including screening for mutations in genes associated with AIDs.

Results: Whole body 123I SAP scintigraphy showed moderate total body amyloid load in both subjects, predominantly involving the spleen and kidneys. SAA and CRP measurements at baseline were markedly elevated. Analysis of the NLRP3 gene by Massively Parallel Sequencing (MPS) revealed mosaic mutations in each case: p.Y563C with mutant allele frequency (MAF) of 11.1% was found in patient 1, and p.A352T with a MAF of 14.6% was detected in patient 2. On direct questioning, clinical symptoms in both patients were fully consistent with CAPS, but with an extraordinarily late onset in life. To explore the origin of the mosaic mutations in our patients, DNA was isolated from a range of hematopoietic and non-hematopoietic samples, revealing varying proportions of the mutant alleles.

Discussion and Conclusion: Systemic AA amyloidosis is a serious potential complication of any inflammatory disorder associated with sustained production of SAA and it has long been recognised that patients with untreated AIDs have a high risk of AA amyloidosis. AA amyloidosis has previously been reported as a complication of CAPS associated with typical germline NLRP3 mutations.

Here we have identified post-zygotic mutational events as the aetiology of late onset CAPS which was complicated by AA amyloidosis in two subjects. The unequal distribution of the variant NLRP3 allele among different cell populations confirmed that the mutational event did not occur in early embryogenesis or before conception. Both of these patients with acquired CAPS responded extremely well to IL-1 blockade, in an identical manner to patients with the usual inherited form.
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AA Cardiomyopathy
N Dasgupta¹, O Cummings², C Phillips², MD Benson²

¹Department of Cardiology, ²Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, Indiana USA

mdbenson@iupui.edu

INTRODUCTION: While amyloid deposits in AA (reactive) amyloidosis can be demonstrated in most organ systems the most common cause of death is renal failure. Clinically significant cardiac involvement with AA is very rare. At the XII International Symposium on Amyloidosis (April 2010) we reported a case of severe AA cardiomyopathy, found to be cause of death at post mortem. We now report two additional cases of cardiac involvement by AA proven by endocardial biopsy.

MATERIAL & METHODS: A 68-year-old Caucasian man with a history of arthritis and multiple joint surgeries presented with proteinuria which led to demonstration of amyloid on kidney biopsy. Shortly thereafter he experienced syncope and fluid retention suggesting congestive heart failure. He was evaluated by Echocardiography and MRI; both were suggestive of amyloid cardiomyopathy. An endocardial biopsy from the right ventricle showed interstitial hyaline deposits consistent with amyloidosis. A second patient, a 63-year-old man, had a heart transplant at age 43 for presumed viral myocarditis. A second heart transplant was required 11 years later due to graft failure. Nine years after the second cardiac transplant the patient developed proteinuria and elevated serum creatinine. A renal biopsy demonstrated amyloid deposition. Endomyocardial biopsy to monitor organ rejection demonstrated perivascular deposits showing green birefringence on polarization of Congo red stained sections consistent with amyloidosis. Further evaluation for systemic disease demonstrated positive rheumatoid factor and joint inflammation consistent with a systemic inflammatory arthritis.

DISCUSSION & CONCLUSIONS: Both of the patients presented here have biopsy proven cardiac amyloidosis. Both also have biopsy proven renal amyloidosis and have medical history consistent with systemic inflammatory disease. In one patient, cardiac functional compromise led to the diagnosis of cardiac amyloid and myocardial biopsy showing interstitial deposition of amyloid is consistent with the amyloidosis being the cause of heart failure. In the second patient the amyloid deposition is mainly perivascular and may not indicate clinically significant cardiac dysfunction. Both patients have evidence of systemic inflammatory disease which was not appreciated by numerous physicians.

AA amyloidosis involvement of the heart is uncommon but certainly should be suspected in any patient with chronic inflammatory disease who presents with abnormalities on echocardiography or MRI suggestive of amyloidosis. Biochemical or histologic typing of the amyloid is imperative.
PC71

Apolipoprotein A-II accelerates reactive AA amyloidosis

M Yang1, Y Liu1, L Li1, H Miyahara1, X Ding1, J Dai1, J Sawashita1,2, M Mori1,2, K Higuchi1,2

1 Department of Aging Biology, Institute of Pathogenesis and Disease Prevention, Shinshu University Graduate School of Medicine, Matsumoto, Japan. 2 Institute for Biomedical Sciences, Interdisciplinary Cluster for Cutting Edge Research, Shinshu University, Matsumoto, Japan.

keiichihi@shinshu-u.ac.jp

INTRODUCTION: During the acute-phase response (APR), serum amyloid A (SAA) circulates in the blood as a high density lipoprotein (HDL). However, other circulating HDL components, ApoA-I and ApoA-II are decreased. The underlying mechanism causing this change in expression pattern is unknown. AA amyloidosis is a disorder characterized by the systemic deposition of SAA fibrils. Our previous research showed that SAA, ApoA-I and ApoA-II, interact with each other during AA amyloid formation in the mouse (1,2). In this study, we used ApoA-II knockout (Apoa2−/−) mice to investigate the potential roles of ApoA-II in lipoprotein particle formation and progress of amyloidosis during experimental AA amyloidosis.

MATERIAL & METHODS: To induce AA amyloidosis, AgNO3 and AA amyloid fibrils were co-injected into wild-type (WT), Apoa1−/−, Apoa2−/− or Apoa2Tg transgenic (Tg) mice. The deposition of AA amyloid (0-10 days) was identified by Congo red-stained sections. The intensity of the AA deposition in each organ was determined using an amyloid score (AS) that was graded from 0 to 4. The amyloid index (AI) is the average of the AS scores in 7 organs (heart, liver, spleen, stomach, intestine, tongue, and skin). Western blot analysis was used to determine plasma SAA levels (0-10 days) with rabbit anti-AA antiserum. Pathological damage in the tissues was determined by tissues sections. Serum pre-stained for lipids with Sudan Black B was electrophoresed on a nondenaturing polyacrylamide gel electrophoresis (PAGE) with a 5-15% polyacrylamide gradient. Lipoprotein particles of pooled sera of mice in some groups were also analysed with a dual-detection, high-performance liquid chromatography (HPLC) system (Liposearch System, Skylight Biotech, Inc., Akita, Japan).

RESULTS: Amyloid deposition was suppressed in Apoa2−/− mice, especially in the spleen, stomach and intestine (Table 1), but accelerated in Apoa2Tg mice. During APR, Apoa2−/− mice showed a decrease in serum SAA levels and hepatic mRNA expression levels of SAA compared with WT mice, while Apoa1−/− mice showed no change. Pathological investigation showed that Apoa2−/− mice experienced less tissue damage and less inflammatory cell infiltration in the lung during APR. The HDL levels of Apoa2−/− mice were markedly decreased but LDL particles increased compared with WT mice. Apoa2−/− and WT mice showed an increase in the lipoprotein levels, mainly LDL and very large HDL during APR.

DISCUSSION and CONCLUSIONS: Our observation demonstrated the potentially important role of ApoA-II in acute phase inflammation and AA amyloidosis morbidity. ApoA-II is associated with aggravated AA amyloidosis. Our previous research showed that the apolipoproteins of HDL have important roles in the induction of AA amyloidosis (2). Although the interaction of ApoA-II and SAA is still not clear, some research has shown that ApoA-II may inhibit the binding of LPS suppression protein and accelerate LPS-induced acute phase inflammation (3). This study may shed light on the relationship between SAA and apoA-II as well as provide new information concerning the mechanism of amyloidosis and its therapy.


<table>
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<tr>
<th>Mouse ID</th>
<th>N</th>
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<tr>
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<td>1.21 ± 0.29</td>
</tr>
<tr>
<td>C57BL/6J.129-Apoa2−/−</td>
<td>7</td>
<td>0.69 ± 0.22</td>
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</table>

Table 1. Amyloid Index after 10 days when mice were treated with AgNO3+AA fibrils (P value=0.003 U-test)
PC72

Incidence rate of Amyloidosis in patients from a medical care program in Buenos Aires, Argentina: A prospective cohort

MA Aguirre, EM Nucifora, DH Giunta, GD Waisman, F Gonzalez Bernaldo de Quiros, ML Posadas-Martinez, BR Boietti

*Department of Medicine, Instituto Universitario Hospital Italiano de Buenos Aires, Argentina. Grupo de Estudio de Amiloidosis, adela.aguirre@hospitalitaliano.org.ar*

**Background**

There is limited data concerning the incidence rate (IR) of AL and AA amyloidosis in the Argentinean population. Our aim was to estimate the IR of AL and AA amyloidosis at the Hospital Italiano Medical Care Program (HIMCP) in Buenos Aires, the most populated city in Argentina.

**Methods**

This cohort study evaluated all consecutive incident cases of AL and AA amyloidosis confirmed in patients over the age of 17 who were members of the HIMCP from January 2006 to December 2014. Any patient who had diagnosis of Amyloidosis and was a member of the HIMCP at the time of diagnosis was considered an incident case.

**Results**

There were 12 and 2 cases of AL and AA Amyloidosis, respectively for 1105152 person-years of follow-up. The crude IR of AL amyloidosis was 11 (95% CI: 6 to 19) and 1.8 (95% CI: 0.5 to 7.2) for AA amyloidosis per 1000000 person-years. The lowest IR was found in women (0.47 and 0.66 for AL and AA 1000000 person-years).

The IRs adjusted to the population of the city of Buenos Aires were 0.58 (95% CI: 0.24 to 0.92) for AL and 1.26(95% CI: 0.5 to 3.1) for AA.

**Conclusions**

This is the first paper to report the cumulative incidence of AL and AA amyloidosis in Latin America. Al and AA amyloidosis is a rare but with high morbid mortality health problem in the HIMCP, especially in men.

**References**

AA amyloidosis in an institutional registry of amyloidosis in Argentina

MA Aguirre, E Nucifora, G Greloni, C Varela, H Garcia Rivello, S Christiansen, R Luxardo, P Sorroche, MS Saez, GD Waisman, F Gonzalez Bernaldo de Quiros, DH Giunta, ML Posadas-Martinez, BR Boietti

Department of Medicine, Instituto Universitario Hospital Italiano de Buenos Aires, Argentina. Grupo de Estudio de Amiloidosis,
adela.aguirre@hospitalitaliano.org.ar

**Background.** AA amyloidosis mainly cause kidney dysfunction leading to an increased risk of mortality in the medium term. The main causes of AA described are rheumatoid arthritis (RA), inflammatory bowel disease (IBD), chronic infections or the cause may not be apparent1.

**Aim.** To describe the epidemiological characteristics of patients with AA amyloidosis in a university tertiary hospital in Argentina.

**Methods.** A prospective clinical cohort, which included all consecutive patients who had evidence of AA amyloidosis confirmed by immunohistochemistry in tissue from April 2012 to January 2016, included in The Institutional Registry of Amyloidosis in the Hospital Italiano de Buenos Aires (RIA - NCT01347047). All data was collected following a standardized evaluation by members of the Hospital Italiano Amyloidosis Group.

**Results:** The Registry enrolled 72 patients, of whom 12 patients met diagnostic criteria for AA amyloidosis. Of all patients with AA, 66.7% (8) were female, with a median age of 52 (RII 38 - 58), 92% (11) had renal involvement, all were diagnosed with nephrotic syndrome, 27.3% (3) had low glomerular filtration rate and 18% (2) required renal replacement therapy permanently. In two cases the diagnosis of amyloidosis preceded the diagnosis of the underlying disease and is reported with negative numbers. The disease that originated AA amyloidosis was 50% (6) of inflammatory origin, in 33% (4) patients suffering from respiratory bacterial repeated infections, in two cases associated bronchiectasis in 16% (2) of patients the cause of AA amyloidosis remains unknown. Time measured in years between the onset of the underlying condition and the diagnosis of AA was in total 10.2 (IQR 0.02-35.1) and 6.6 (IQR 2.4 - 24.3) and 22.5 (IQR 0.1 - 35.1) for infectious diseases and inflammatory origin, respectively. The longest latency period measured in years since the beginning of the underlying disease to diagnosis of amyloidosis was 45 years.

**Discussion.** In our country there are still no epidemiological data of AA amyloidosis. Knowing the distribution of causes of AA amyloidosis and characteristics in our region is important for the scientific and health community. The causes of AA amyloidosis in our center is similar to those of developed countries, probably due to the profile of the population attending at our institution2. The most commonly affected organ is the kidney as it is widely described in the literature. Particularly striking is the difference in time between the onset of symptoms of the underlying disease and the onset of clinical amyloidosis in the case of rheumatic diseases this latency is measured in decades and in infectious diseases is measured in years. In this regard it would be useful to have means for detecting SAA (serum amyloid A) to confirm this observation.

Amyloid and an interested service rheumatologist
– A strengths, weaknesses, opportunities & threats review

EA Clarke¹, T Akhtar¹, J Hunter¹

¹Rheumatology Department, Gartnavel General Hospital, Glasgow, G12 0YN, Scotland

Introduction: Amyloidosis is part of the curriculum for rheumatologists in training in the UK state-funded health service. Exposure in training and as a rheumatology specialist is generally limited. We examined the work involved in running a small specialist clinic, have considered possibilities for development, and would value comments.

Methods: Notes of all patients attending the amyloid service over the course of 1 year, from 28/2/15 to 29/2/16 were reviewed and the work involved documented.

Results: Over the course of 1 year, 26 patients were referred to an amyloid clinic and 24 attended for follow up. Of the 50 patients, 22% were referred by rheumatology, 20% by neurology and 18% by renal. Other referrals came from respiratory, haematology, orthopaedics, infectious diseases, primary care, dermatology and cardiology. Only 18% of patients came from the geographical area (population approximately 200,000) usually covered by the rheumatology service at the base hospital.

Patients had a mean of 2.3 visits/year. 19 had only 1 visit, 11 of whom were discharged without amyloid being demonstrated. 2 patients (one each with AL and ATTR amyloid) were the subject of correspondence but did not attend. The total number of appointments was 95. Email correspondence between related specialties and the clinic service averaged 13/month, but most correspondence was by letter.

The diagnoses in the 50 patients were systemic AL amyloid (n=7, 14%); AA (n=11, 22%); ATTR (n=5, 10%); 1 patient with variant fibrinogen A alpha chain amyloidosis; and localised forms of amyloid (including nodular pulmonary and multifocal skin amyloid) were present in 9 patients (18%). 2 patients attend with periodic fever syndromes, but neither has amyloid. 1 additional patient has evidence of cardiac amyloid but no pathology results yet; 1 has no diagnosis as the patient chose to defer investigation; 13 patients had no evidence of amyloid despite biopsies and were discharged. There were 2 deaths within the cohort: 1 patient with ATTR had a myocardial infarction and 1 with a diagnosis of AA amyloid died of sepsis.

Within the new referrals group from this period, 12 patients do not have a diagnosis of amyloid (46%); 2 have unclear diagnoses due to deferred tests (1) or imaging suggestive of cardiac amyloid but no pathology yet (1). Of the remaining 12 new patients, diagnoses are: AA amyloid 2 (7% of total new patients); AL amyloid 6 (23%); localised forms of amyloid 3 (12%); and ATTR amyloid 1 (4%).

Clinic based diagnostic testing included 18 abdominal fat aspirates during the year, of which 4 showed amyloid. Some patients with a negative fat aspirate were diagnosed with amyloid via samples from other tissues, generally taken by other specialties (orthopaedics, dermatology, and gastroenterology).

23 of the 50 patients (46%) attended the UK National Amyloidosis Centre and a further 15 had biopsy samples examined there. 25% of patients currently only attend our hospital for their amyloid and related problems; 75% continue to see another specialty because of problems related to amyloid – most commonly renal and rheumatology.

Discussion: The strengths of a regional service include training opportunities, good links to relevant related specialties and primary care (a multidisciplinary approach), opportunities for others to discuss “possible” amyloidosis, and an opportunity for patients to ask questions locally. The clinic runs in a day ward setting which allows access to supportive nurses and day patient biologic therapies. Some patients also decline the round trip (over 1100km) to the UK centre. Weaknesses are the involvement of only 1 consultant, difficulties in service development in a cash-strapped state health service, and only a minority of patients having an associated rheumatological diagnosis. These issues demonstrate the opportunities and threats for the future. It may be useful if other specialties choose to run an amyloid service.
PC75

A simple test for AA amyloid
T Yamada, J Sato

Department of Clinical Laboratory Medicine, Jichi medical University, Shimotsuke, Japan

yamadanji@jichi.ac.jp

INTRODUCTION:
Detection of AA amyloid in the biopsy materials is not difficult with the use of histological methods. However, there is some material like abdominal fat aspirates, which are not easy to process into histology specimens. Here, we introduce a simple method for suspecting the presence of AA amyloid.

MATERIAL & METHODS:
Two monoclonal antibodies, anti-SAA30 and anti-SAA84, were utilized [1]. The number indicates the epitope region in SAA(serum amyloid A) molecule recognized by each antibody. Since AA amyloid usually defects the carboxyl terminal region beyond the residue 77, the positive reaction with anti-SAA30 and the negative reaction with anti-SAA84 may suggest the presence of AA amyloid. The antibodies were conjugated to gold colloid particle and suspended in saline-based buffer. The biopsy materials were sonicated in a 4M guanidine HCl buffer, pH 8.2 and mixed 1: 10 with the antibody-particle solution. Five minutes later, the aggregation was read optically or measured by spectrophotometer at 492 nm.

RESULTS:
The detection sensitivity for both the systems was approximately 10 ng/mL when rSAA76 was used as the sample. When reacted with anti-SAA30 solution (Fig. 1) and was 10 ng/mL when recombinant SAA was reacted with anti-SAA84 solution. Gastric mucosa samples from AA amyloidosis patients resulted in positive with anti-SAA30 and negative with anti-SAA84.

DISCUSSION & CONCLUSIONS: The test was simple and easy to use. The combined use of the two antibody system may avoid the contamination of SAA-high blood. More accurate identification would require the immunoblot, which could indicate the size of the AA proteins [2].

REFERENCES:

Fig. 1: Aggregation of anti-SAA30 and anti-SAA84 particles reacted with sonicated amyloidotic materials.
N-terminal region of serum amyloid A3 is responsible for up-regulation of MUC2 mRNA expression in mouse epithelial cells

Yasuo Inoshima1,2, Manami Tashiro1, Naotaka Ishiguro1,2

1 Laboratory of Food and Environmental Hygiene, Department of Veterinary Medicine, Gifu University, Gifu, Japan. 2 United Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan.

INTRODUCTION: In both humans and animals, serum amyloid A (SAA) is known as a precursor protein of amyloid A (AA) in AA amyloidosis. In mice, SAA is classified into several isoforms such as SAA1, 2, 3, and 4 according to different nucleotide sequences. Although the biological functions of SAA isoforms are not completely understood, previous studies have reported that expression of SAA3 on the mouse colon surface in vivo is increased in the presence of microbiota, suggesting that SAA3 plays a role in host defense. Recently, we demonstrated that in murine colonic epithelial cells, SAA3, but not SAA1, significantly up-regulates mRNA expression of mucin 2 (MUC2); mucins constitute a protective mucus barrier in the intestinal tract. However, the differences between SAA3 and the other SAAs as well as the mechanism for the up-regulation of MUC2 expression remain unclear. The aim of this study is to identify the regions in SAA3 responsible for MUC2 expression.

MATERIALS & METHODS: Recombinant murine SAA1 (rSAA1) and rSAA3 as well as their chimera proteins (rSAA1/3 and rSAA3/1) were produced by Escherichia coli. These rSAAs were added to mouse colonic epithelial CMT-93 cells, and mRNA expressions of MUC2 were examined by quantitative real-time PCR. mRNA expressions of regenerating islet-derived 3 (REG III)-γ, α-defensin (Def), β-Def-3, and β-Def-4, all of which are anti-bacterial proteins secreted from intestinal epithelial cells after sensing the presence of bacteria, were also examined. Inhibition assays using NF-κB and toll-like receptor 4 (TLR4)/MD2 inhibitors were performed.

RESULTS: MUC2 mRNA expression was significantly up-regulated by rSAA3 and rSAA3/1 compared to that by rSAA1 and rSAA1/3. mRNAs of the other anti-bacterial proteins, REG III-γ, α-Def, β-Def-3, and β-Def-4, were not affected by rSAAs. In addition, both NF-κB and TLR4/MD2 inhibitors suppressed MUC2 mRNA expression by rSAA3 and rSAA3/1, respectively.

DISCUSSION & CONCLUSIONS: These results suggest that the region responsible for MUC2 expression is present in the N-terminal region of SAA3, and the up-regulation of MUC2 expression by SAA3 is involved in the activation of NF-κB via the TLR4/MD2 complex.

REFERENCES:
SIGNIFICANT ASSOCIATION BETWEEN RENAL FUNCTION AND AREA OF AMYLOID DEPOSITION EVIDENT IN KIDNEY BIOPSY SPECIMENS IN BOTH AA AND AL AMYLOIDOSIS

T. Kuroda¹, M Ueno², Y. Nozawa¹, H. Sato¹, T. Nakatsue¹, Y. Wada¹, M. Nakano³, I. Narita¹

¹Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan. ²University Health Center, Joetsu University of Education, Niigata, Japan. ³Department of Medical Technology, School of Health Sciences, Faculty of Medicine, Niigata University, Niigata, Japan.

INTRODUCTION: The kidney is a major target organ for systemic amyloidosis. Proteinuria and elevated levels of serum creatinine are present in the majority of affected individuals. Patients that present with amyloid A or amyloid AL show different clinical manifestations. Renal damage due to amyloid deposition also appears to be distinct. The purpose of this study was to clarify the correlation between the area occupied by amyloid in renal biopsy specimens and clinical parameters in both AA and AL amyloidosis.

MATERIAL & METHODS: One-hundred and sixteen patients participated in this study in which 58 patients had an established diagnosis of reactive AA amyloidosis with rheumatoid arthritis (AA group) and 58 patients had AL amyloidosis (AL group). We retrospectively investigated the correlation between clinical data and amyloid occupied area in whole renal biopsy specimens. For statistical analyses, %amyloid-positive areas were transformed to common logarithmic values (Log10 %amyloid).

RESULTS: Irrespective of the amyloid group type, all patients showed amyloid deposits in renal tissues. In most of the specimens, the percentage area occupied by amyloid was less than 10%. By the comparison of clinical characteristics of AA and AL amyloidosis patients, systolic blood pressure was significantly higher in the AA group. In addition, urinary protein, creatinine clearance (Ccr), and glomerular filtration rate (eGFR) were significantly higher in the AL group. As for correlation between Log10%amyloid and renal function, Ccr, and eGFR showed a significant negative correlation with Log10%amyloid in both groups, whereas Cr and blood urea nitrogen showed a significant positive correlation only in the AA group. Urinary protein was not significantly correlated with Log10%amyloid in both groups. Results of sex-, age-, and Log10%amyloid-adjusted multiple linear regression analyses in both groups showed that systolic blood pressure was higher in the AA group than in the AL group. Red blood cell count and hematocrit were significantly lower in the AA group. In relation to renal function, Cr, Ccr, and eGFR all indicated significant impairment in the AA group, whereas urinary protein indicated significant impairment in the AL group. In the renal pathological findings, amyloid in the AL group was significantly deposited in the glomerular capillary wall, whereas in the AA group, amyloid was deposited in the arteriole walls and small artery significantly. In addition, perimembranous type of amyloid deposition was frequently observed in the AL group and nodular type of amyloid deposition in the mesangium area was frequently observed in the AA group.

DISCUSSION & CONCLUSIONS: There are significant differences between AA and AL amyloidosis with regard to the association between the amyloid-positive area in renal tissue and renal function, especially in terms of Ccr, eGFR and urinary protein. These differences could be attributed to the pattern of amyloid deposition in the kidney.

Structure of serum amyloid A suggests a mechanism for selective lipoprotein binding and functions: SAA as a hub in macromolecular interaction networks

N Frame, O Gursky

Department of Physiology & Biophysics, Boston University School of Medicine, Boston USA.
Gursky@bu.edu

INTRODUCTION: Serum amyloid A is a major acute-phase plasma protein that causes AA amyloidosis, contributes to cardiovascular disease, and is a clinical marker of inflammation. SAA N-terminal fragments deposit in AA amyloidosis, a life-threatening complication of chronic inflammation. SAA modulates immune response, cholesterol homeostasis and cell signaling, yet its normal functions remain elusive. In acute injury or infection, plasma levels of SAA can increase >1000-fold reaching >1 mg/ml. Intriguingly, the advantage for survival of this dramatic increase is unclear; it is thought to result, in part, from the ability of SAA to mobilize cholesterol for cell repair. Most circulating SAA binds to high-density lipoproteins (HDL) and re-routes cholesterol metabolism by interacting with cell receptors such as CD36. SAA also interacts with an array of diverse ligands (cholesterol, retinol, heparan sulfate proteoglycans, metal ions, etc.) This binding promiscuity complicates functional studies of SAA often rendering them controversial.

MATERIALS & METHODS: We combine bioinformatics analysis of SAA family members with the recently determined x-ray crystal structures of human and murine proteins to identify a novel binding site that is selective for HDL. The results prompt us to propose a new concept: SAA acts as a protein hub in interaction networks including various proteins, lipids and proteoglycans.

RESULTS: The first structural model of lipoprotein-bound monomeric SAA is proposed wherein two amphipathic alpha-helices from the N-terminal 69-residue domain form a concave hydrophobic surface that binds lipoproteins. The curvature of this surface, which is maintained through close helical packing via the conserved GPGG motif, confers binding selectivity for HDL vis a vis larger lipoproteins. Such packing represents a novel structural motif. A ~30-residue C-domain, connected to the N-domain via a flexible linker, binds various polar/charged ligands including cell receptors such as CD36, bridging them with HDL and thereby re-routing cholesterol transport for cell repair.

DISCUSSION & CONCLUSIONS: Our model is supported by the SAA cleavage in the inter-domain linker, which starts at residue 70, to generate the 1-76 fragment that deposits in AA amyloidosis. The presence of multiple SAA copies on the same HDL particle amplifies the molecular-hub action of SAA. This action is consistent with the causative link between inflammation and atherosclerosis and may shed light on other functions of this enigmatic protein in health and disease.

REFERENCES:


Fig. 1 Structural model of SAA bound to an HDL particle. SAA monomer binds HDL via the concave apolar site (gold), which contains helices 1 and 3 from the N-domain (residues 1-69). Such binding protects SAA amyloidogenic segments from misfolding. The dynamic C-domain, which is cleaved in AA amyloidosis, binds cell receptors bridging them with HDL.
New atomic insights into the mechanism underlying apolipoprotein AI-associated amyloidosis.

D Townsend¹, D A Middleton¹

¹ Department of Chemistry, Lancaster University, Lancaster, UK.
d.townsend1@lancaster.ac.uk

Assembly of apolipoprotein AI (Apo AI), the main protein component of high-density lipoprotein (HDL), into amyloid species can reduce HDL’s atheroprotective mechanism, increase atherosclerotic plaque loading and produce excessive inflammation caused by cytotoxic fibril intermediates. The N-terminus of wild-type Apo AI (residues 1-93) and various mutants are associated with its aggregation into amyloid and deposition in the heart, liver and kidney (1). Here we use biophysical techniques to investigate the mechanism of Apo AI aggregation. Apo AI is stable at pH 7, but at pH 4, used to mimic acidic environments experienced in atherosclerotic plaques due to the release of arachidonic acid (2) and the effects of fatty acid release (3), ApoA-I rapidly assembles into ThT responsive species, and the rate of this process, and fibril yield, is enhanced in the presence of heparin, a proxy for glycosaminoglycans that are found associated with virtually all amyloid in vivo. The aggregates formed in the presence of heparin have the appearance of amyloid-like fibrils by electron microscopy, bind to the amyloid inhibitor epigallocatechin gallate and give rise to characteristic 10 Å and 4.7 Å spacing in the X-ray diffraction pattern. Circular dichroism (CD) spectra of Apo AI in solution are consistent with rapid precipitation of the protein (after incubation for less than 1 h at pH 4), but CD spectra of dried films of the insoluble precipitate suggest that the aggregates retain a high degree of the native α-helical structure. Solid-state NMR spectra of lyophilized ApoA-I at pH 7 shows strong agreement with the established helical structure of lipid-free ApoA-I, as reported by Ajees (4), but spectra of the precipitates are consistent with a structural transition to a unique amyloid form containing both β-sheet and α-helical structures, both in the presence and absence of heparin. From a series of NMR restraints we propose that the N terminus (1-40) of ApoA-I mis-folds into amyloid species, whilst the C terminus retains its native α-helical content. A simulated spectrum of this model, containing residues 1-40 in a β-sheet conformation and residues 41-236 in the native α-helical conformation, agrees with the NMR spectrum and as such, supports this model. These results will also help to understand why over 10 mutants of Apo AI are associated with hereditary amyloidosis.

REFERENCES:

Hereditary systemic amyloidosis due to a novel amyloidogenic Apo25Val apolipoprotein C-III variant

N Jourde-Chiche1, F Bridoux2, B Nédelec3, JM Goujon4, A Dogan5,6, A Mangé2, A Kontush6, P N Hawkins8, J D Gillmore6, V Bellotti9,10, P P Mangione9,10, M Chabert11, A Kontush8, P N Hawkins9, J D Gillmore9, V Bellotti9,10, P P Mangione9,10, M Chabert11, A Kontush8, I Nédelec3, S Valleix3,12

1Service de Néphrologie, AP-HM, Hôpital de la Conception, Marseille, France. 2Service de Néphrologie et de transplantation rénale, Université de Poitiers, Centre national de référence amylose AL et autres maladies par dépôts d’immunoglobulines monoclonales, Poitiers, France. 3Institut Imagine, INSERM UMR_1163, Université Paris-Descartes, Sorbonne Paris Cité, Paris, France. 4Service d’anatomie et de cytologie pathologiques, CHU Poitiers, France. 5Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55901, USA. 6Department of Laboratory Medicine and Pathology, Memorial Sloan-Kettering Cancer Centre, New York, NY 10065, USA. 7Institut de Recherche en Cancérologie de Montpellier (IRCM), INSERM, U1194, Montpellier 34298, France. 8Sorbonne Université, UPMC Univ Paris 06, Institute of Cardiometabolism and Nutrition (ICAN), UMR_S 1166, Hôpital de la Pitié, Paris 75013, France. 9Centre for Amyloidosis and Acute Phase Proteins, National Amyloidosis Centre, University College London, London NW3 2PF, UK. 10Department of Molecular Medicine, Institute of Biochemistry, University of Pavia, Via Taramelli 3b, Pavia 27100, Italy. 11Sorbonne Université, UPMC Univ Paris 06, INSERM, Université Paris-Descartes, Sorbonne Paris Cité, UMR_S 1138, Centre de Recherche des Cordeliers, Paris 75006, France. 12Laboratoire de Génétique Moléculaire, Hôpital Necker-Enfants Malades, AP-HP, Université Paris-Descartes Sorbonne Paris Cité, Paris, France. sophie.valleix@aphp.fr

INTRODUCTION:

We report the identification of a new amyloid protein in humans, apolipoprotein C-III, responsible for a novel form of autosomal dominant amyloidosis with sicca syndrome and severe renal dysfunction. Identification of the amyloidogenic Asp25Val apoC-III variant started from the observation of an unexpected cardioprotective lipid profile in all affected members of this family, characterized by hypotriglyceridemia, a dramatic decrease in VLDL particles with a concomitant major increase in HDL fraction.

MATERIAL & METHODS:

The exons and the promoter region of APOC3 were PCR amplified and sequenced by Sanger method in all available family members. Amyloid was detected by Congo red staining, indirect immunofluorescence and immunoelectron microscopy were performed with a polyclonal antibody against the apoC-III protein. LMD/MS proteomics was performed from salivary glands, heart and digestive tract. Lipid and lipoprotein analysis used standard biochemical assays, and sequential ultracentrifugation.

RESULTS:

The French family consisted of 8 affected members over four generations with amyloid deposits in salivary glands, kidney and other numerous organs for which the subtyping remained inconclusive. The initial genetic screening of all genes known to be involved in hereditary amyloidosis was also negative. The APOC3 gene was sequenced on the basis of hypotriglyceridemia and a low VLDL/high HDL level, a biological profile uncommon in the context of chronic renal failure. A new Asp25Val variant of apoC-III was found to co-segregate with the amyloid status and the cardioprotective lipid profile. Amyloid subtyping was performed by immunofluorescence, immunogold staining and LMD/MS-based proteomics from several affected tissues of six different patients, confirming that amyloid fibrils were composed of the variant Asp25Val apoC-III only. The affected members of this kindred had decreased levels of apoC-III in plasma and on HDL particles with a twofold reduction of Asp25Val apoC-III compared to the wild-type apoC-III. As a consequence, VLDL-TG hydrolysis proceeds at high rate provoking the drastic decreased in VLDL and the increase in HDL particle number. We hypothesize that the preferential incorporation of Asp25Val apoC-III into amyloid fibrils is likely the main cause of the imbalance in the ratio of wild-type/Asp25Val apoC-III on HDLs.

DISCUSSION & CONCLUSIONS:

The Asp25Val apoC-III variant is the first missense variant of APOC3 to be amyloidogenic as well as conferring cardioprotection even in the unfavorable context of severe renal failure. In an attempt to slow down amyloid formation in these patients, fibrates that significantly reduce hepatic APOC3 transcription may have therapeutic potential for this new form of hereditary amyloidosis.
INTRODUCTION:

Naturally occurring mutations of Human apolipoprotein A-I (apoA-I) have been shown to induce amyloidosis in patients, with a broad range of clinical manifestations, depending on the protein variant which is involved (1). Although the molecular mechanisms of apoA-I amyloid associated pathology remain largely unknown, the fact that the wild-type (Wt) deposits in atherosclerotic plaques supports the hypothesis that a chronic inflammatory micro environment could elicit protein aggregation (2).

In order to get insight into the mechanisms inducing apoA-I misfolding, we examined the effects of point mutations in apoA-I on the structure, stability, and aggregation propensity, as well as on the ability to bind to putative ligands.

MATERIAL & METHODS:

Proteins were incubated under different conditions (low pH, in the presence of ligands, proteolysis enzymes etc) and structural features associated to protein folding were analyzed by fluorescence spectroscopy and western blotting. Aggregates were characterized by Atomic Force Microscopy. Pro-inflammatory Cell activation was checked by using standard macrophage cell cultures (3)

RESULTS:

Our results indicate that all the mutants tested (Gly26Arg, Lys107-0, Arg173Pro and Leu60Arg) are less stable than the Wt. Arg173Pro shows a higher susceptibility to partial proteolysis, and a lower efficiency to bind to phospholipid vesicles at physiological pH, which could determine the observed higher tendency to aggregate as pro-amyloidogenic complexes. But in addition, this mutant binds more efficiently to heparin or other ligands, even at physiological pH, which could explain its retention in the pro-inflammatory landscape. Lys107-0 shows higher tendency to aggregate than Gly26Arg, but, interestingly Gly26Arg and Arg173Pro (but not Lys107-0) are able to elicit macrophage activation, thus stimulating local chronic inflammation.

DISCUSSION & CONCLUSIONS

Our results suggest that apoA-I mutants share some but not all the same mechanism of pathogenicity. More than stability, binding to ligands as heparin or other glycosaminoglycans could be key events tuning the fine details of the interaction of apoA-I variants with the micro-environment. Moreover, induction of macrophage activation could be an attractive hypothesis to explain why certain variants contribute more than others to apoA-I-induced pathogenesis. Further studies should focus on the complex landscape mediating the roles of apoA-I in the delicate balance between health and pathology.

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LECT2 AMYLOIDOSIS: NOT JUST A HISPANIC DISEASE

Simon Gibbs\textsuperscript{1,2,4}, Fiona Kwok\textsuperscript{2,4}, John Ramzy\textsuperscript{1}, Janet Gilbertson\textsuperscript{5}, Graeme Stewart\textsuperscript{2,4}, Peter Mollee\textsuperscript{3,4}

1 – The Victorian and Tasmanian Amyloidosis Service, Melbourne;

2 – The Westmead Amyloidosis Clinic, Sydney; 3 – The Princess Alexandra Hospital Amyloidosis Centre, Brisbane; 4 – The Australian Amyloidosis Network

5 – The National Amyloidosis Centre, UCL Medical School, London, UK

simon.gibbs@monash.edu

Introduction: Leukocyte chemotactic factor 2 amyloidosis (ALECT2) can mimic AL amyloidosis. First described in 2008, ALECT2 involves the kidneys and liver and 90% of reported cases are Hispanic. With proteomics, 196 cases are described and it is the third most common renal amyloidosis in USA. No Australian cases are reported. We sought to describe all patients diagnosed with ALECT2 in Australia.

Method: Members of the Australian Amyloidosis Network was contacted to submit details of patients diagnosed with ALECT2. Patient age, ethnicity, renal function, disease characteristics, patient outcome and diagnostic methods were collected.

Results: Three cases were identified. Case 1. 49-year-old Egyptian man with hypertension. Case 2. 65-year-old Egyptian man with hypertension. Case 3. 65-year-old man of Indian British heritage. All patients presented with incidental worsening chronic kidney disease without proteinuria. All had normal liver function tests, cardiac biomarkers, serum free light chains and no paraprotein. Renal biopsies were examined with light microscopy, Congo red staining, immunohistochemistry and electron microscopy (EM). Patients had amyloidosis gene testing for mutations of transthyretin, lysozyme, fibrinogen A-α chain and apolipoprotein A1- all negative.

Case 1 and 3 renal biopsies showed amyloid deposition on light microscopy with Congo red. Case 2 biopsy demonstrated hypertensive nephropathy and EM confirmed amyloid. Kappa, lambda and AA immunohistochemistry was negative in all. Biopsies from case 1 and 3 underwent laser capture microdissection and tandem mass spectrometry, confirming ALECT2. Immunohistochemistry with anti-LECT2 antibody was positive for all. Renal function remained stable in two, no patient died.

Discussion and Conclusion: We present the first three Australian cases of ALECT2. It is important to distinguish ALECT2 from AL amyloidosis to avoid inappropriate chemotherapy. ALECT2 has a good prognosis and should be considered in cases of renal amyloidosis without detectable plasma cell dyscrasias and negative hereditary screen - especially in Hispanics or Egyptians. Mass spectrometry and LECT2 immunohistochemistry are valuable diagnostic tools.

Figure 1. Renal Biopsy from Case 1. (Left) Congo red + polarised light (magnification x100), the material has green birefringence (Right) Immunohistochemical analysis using goat anti-human LECT monoclonal antibody (original magnification x100).
Structure, function and aggregation propensity of the novel amyloidogenic D25V apolipoprotein C-III variant

G Verona1,2, R Porcari2, CA Waudby3, A Relini4, PJ Talmud5, O Kovrov6, G Olivecrona6, J Christodoulou1, JD Gillmore1, PN Hawkins1, S Valleix7, V Bellotti1,2, PP Mangione1,2

1 Centre for Amyloidosis and Acute Phase Proteins, University College London, London, UK. 2 Department of Molecular Medicine, University of Pavia, Pavia, Italy. 3 Institute of Structural and Molecular Biology, University College London, London, UK. 4 Department of Physics, University of Genoa, Genoa, Italy. 5 Centre for Cardiovascular Genetics, University College London, London, UK. 6 Department of Medical Biosciences, Umeå University, Umeå. 7 Laboratoire de génétique, Hôpital Necker-Enfants malades, Institut IMAGINE_INSERM U1163, Université Paris-Descartes, Paris, France.

g.verona@ucl.ac.uk

INTRODUCTION: The first amyloidogenic variant of apolipoprotein C-III (apoC-III), D25V, has been recently identified in a French family causing both severe renal amyloidosis and low levels of plasma triglycerides [1]. In its lipid bound state, apoC-III shares the same structural motif of six amphipathic α-helices as other exchangeable apolipoproteins. In their lipid free form they have lower conformational stability and some are prone to self-assembly as amyloid fibrils. We have investigated the effect of the mutation on structure and function of D25V variant apoC-III in vitro and characterize its propensity to form amyloid fibrils in its lipid-free form.

MATERIALS & METHODS: Comparative structural analyses of recombinant wild type and D25V apoC-III were performed using both predictive tools and spectroscopic techniques including circular dichroism and NMR. Fibrillogenesis of the variant was carried out in physiological conditions and monitored by thioflavin T emission fluorescence, turbidity, electron microscopy and atomic force microscopy. Functional properties, such as lipid binding and inhibition of lipoprotein lipase (LPL), were investigated for both wild type and variant apoC-III in vitro.

RESULTS: The D25V mutation enhances both the beta-sheet content and aggregation propensity in the apoC-III polypeptide. The variant protein aggregates in physiological conditions and fluid agitation with rapid formation of genuine amyloid fibrils whereas the wild type apoC-III maintains its soluble native state. Functional studies show that the effect of the mutation may cause a less efficient lipid binding compared with the wild type whereas it does not alter the inhibition of the LPL activity. NMR analysis of the two proteins in the presence of sodium dodecyl sulfate reveals that the minimal changes in the structure are clustered around the site of the mutation.

DISCUSSION & CONCLUSIONS: The discovery and characterization of the D25V apoC-III variant confirms that amyloidogenic lipoproteins are components of high density lipoproteins and that they are prone to amyloid fibril formation in their lipid free state.

REFERENCES:
A specific nanobody prevents amyloidogenesis of D76N β2-microglobulin *in vitro* and modify its tissue distribution *in vivo*

S Raimondi1, R Porcari2, G Verona1,2, S Ellmerich2, G Faravelli1, S Giorgetti1, L Marchese1, I Zorzoli3, A Gallanti1, G Esposito4,5, JP Simons2, R Al-Shawi6, J Marcoux7, GW Taylor2, PP Mangione1,2, V Bellotti1,2

1 Department of Molecular Medicine, University of Pavia, Pavia, Italy. 2 Centre for Amyloidosis and Acute Phase Proteins, University College London, London, UK. 3 Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy. 4 Department of Medical and Biological Sciences, University of Udine. 5 Science and Math Division, New York University at Abu Dhabi, Abu Dhabi, UAE. 6 Centre for Biomedical Science, Division of Medicine, University College London, London, UK. 7 CNRS, Institute of Pharmacology and Structural Biology, Toulouse, France.

sara.raimondi@unipv.it

INTRODUCTION: Systemic amyloidosis is caused by misfolding and polymerization of globular proteins. Mutations, selective truncation, abnormal and persistent increase in plasma concentrations are the events driving the amyloid transformation of globular proteins. New effective treatments are urgently needed and the inhibition of protein polymerization represents a putative promising target. Amyloid conversion of β2-microglobulin (β2m) has been extensively investigated [1]. Here we focus our studies on the inhibition of the amyloidogenic natural D76N variant [2], that is associated with hereditary familial amyloidosis and forms fibrils in physiological conditions [3].

MATERIAL & METHODS: The inhibitory activity of doxycycline, rifamycin and Nb24 nanobody [4] on D76N β2m fibril formation was assessed by a combination of techniques including thioflavin T assay and/or quantification of the soluble fraction. Electron microscopy and light microscopy under polarized light after Congo red staining were also used to monitor amyloid formation. Organ distribution in mice was determined after intravenous injection of 125I-D76N or 125I-wild type β2m in the presence and in the absence of Nb24.

RESULTS: We have compared the potency of three previously described inhibitors of β2m fibrillogenesis: doxycycline, rifamycin and Nb24 nanobody [4] on D76N β2m fibril formation was assessed by a combination of techniques including thioflavin T assay and/or quantification of the soluble fraction. Electron microscopy and light microscopy under polarized light after Congo red staining were also used to monitor amyloid formation. Organ distribution in mice was determined after intravenous injection of 125I-D76N or 125I-wild type β2m in the presence and in the absence of Nb24.

DISCUSSION & CONCLUSIONS: These findings support the perspective of a direct therapeutic use of this antibody which could not only impede the fibrillar aggregation, but can also reduce the concentration of the variant in the extracellular space. The latter effect is not observed with wild type β2m and therefore we may assume that the misfolded variant accumulates in the extracellular matrix and that the antibody may reduce its deposition by stabilizing the fully folded form of this highly amyloidogenic protein.

REFERENCES:

Renal amyloidosis associated with four novel variants in the fibrinogen a-alpha chain protein.

Dorota Rowczenio1, Maria Stensland2,3, Gustavo Antonio de Souza2,3, Erik H. Strøm2,5, Janet A Gilbertson1, Nigel Rendell1, Graham Taylor1, Ketil Riddervold6, Kristian Selvig7, Inger Karin Lægreid8, Philip N Hawkins1, Tale Norbye Wien2,8 and Julian D. Gillmore1.

1National Amyloidosis Centre, Division of Medicine, Royal Free Campus, UCL, London, UK. 2Norwegian Work group of Amyloidosis, Department of Pathology, Oslo University Hospital Rikshospitalet, Oslo, Norway. 3Proteomic Core Facility, Department of Immunology, Oslo University Hospital Rikshospitalet and University of Oslo, Oslo, Norway. 4Department of Pathology, Oslo University Hospital Rikshospitalet, Oslo, Norway. 5Department of Genetics, Oslo University Hospital Rikshospitalet, Oslo, Norway. 6Department of Internal Medicine, Drammen Hospital, Drammen, Norway. 7Department of Nephrology, St. Olavs Hospital, Trondheim, Norway. 8Department of Internal Medicine, Bærum Hospital, Bærum, Norway

d.rowczenio@ucl.ac.uk

Introduction: Fibrinogen A alpha-chain (AFib) amyloidosis is an autosomal dominant disease associated with mutations in the fibrinogen A alpha-chain (FGA) gene. Patients typically present with kidney impairment and progress to ESRD over a median time of 4.6 years from onset of symptoms. Here we report five novel mutations in FGA gene; four were associated with renal amyloidosis and one was determined to be an incidental finding in a patient with AL amyloidosis.

Methods: Five unrelated patients underwent detailed clinical evaluation after amyloid deposits were discovered on kidney biopsy. Laser Micro Dissection (LMD) followed by liquid chromatography and tandem Mass Spectrometry (MS) was performed on amyloidotic glomerular samples. Genes associated with hereditary renal amyloidosis were analyzed by Sanger sequencing.

Results: All patients presented with proteinuria, hypertension and /or lower limb oedema. In one case the kidney biopsy revealed Congo red staining in the glomeruli and in vessels, but immunohistochemical typing of the amyloid was inconclusive. Subsequently, bone marrow examination revealed a clonal plasma cell population and LMD and MS identified kappa light chains as the amyloid fibril protein. In the remaining four subjects extensive amyloid deposits were found solely within the glomeruli, which stained specifically with antibodies to fibrinogen Aα-chain; monoclonal protein studies were negative. Interestingly, one of the four latter patients also had glomerular crescents and high titre anti-GBM antibodies, and was diagnosed to have co-existing anti-GBM glomerulonephritis.

A novel FGA gene mutation was identified in each case: two patients had frame shift mutations F521Sfs*27 and G519Efs*31, whilst the other subjects had substitutions resulting in G555F, E524K and R554H variants.

Discussion and Conclusion: To date nine FGA variants have been identified in different kindreds and although the precise mechanism for amyloidogenicity in this disease is not fully understood, it has been shown that all mutations are clustered within close proximity in the 5’ end of exon 5 of the FGA gene. Here we report five patients with novel FGA mutations. In four cases the diagnosis of AFib amyloidosis was confirmed on a kidney biopsy using a combination of immunohistochemistry and LMD and MS, in conjunction with genetic analysis. The renal histological appearance in all patients with AFib amyloidosis in whom the novel FGA variants were found was identical to all previously reported amyloidogenic FGA variants with characteristic massive glomerular amyloid in the absence of extraglomerular deposits. Interestingly, the kidney biopsy in one subject revealed the presence of characteristic glomerular fibrinogen amyloid deposits as well as crescentic glomerulonephritis, the latter in association with high titre anti-GBM antibodies. To our knowledge he is the first case diagnosed with AFib amyloidosis and anti-GBM antibody disease.

The novel R554H substitution was not associated with AFib amyloidosis. The patient was diagnosed with AL amyloidosis and serial SAP scintigraphy showed regression of amyloid and improvement in amyloidotic organ dysfunction following chemotherapy.

Clinical awareness and suspicion of hereditary amyloidosis corroborated by genetic analysis and adequate typing using combined immunohistochemistry and LMD and MS, especially in the absence of a family history of amyloidosis, is essential to avoid misdiagnosis that could lead to administration of inappropriate and potentially harmful therapy.
The importance of pre-clinical studies in animal models of TTR amyloidosis for the discovery of novel patient disease biomarkers

NP Gonçalves1, D Martins1,2, MJ Saraiva1,2

1Instituto de Inovação e Investigação em Saúde (I3S), Universidade do Porto, Portugal; 2 Unidade de Neurobiologia Molecular, Universidade do Porto, Portugal.

mjsaraiv@ibmc.up.pt

INTRODUCTION: It was previously demonstrated that an inflammatory stimulus in the peripheral nerve of TTR V30M transgenic mice is able to trigger and precede TTR V30M deposition, contributing for the positive feedback loop of toxicity and Familial Amyloidotic Polyneuropathy (FAP) progression. MATERIAL & METHODS: To investigate the molecular mechanisms underlying this phenomenon we performed a whole-mouse genome Agilent microarray analysis of damaged V30M nerves as compared with those from WT mice.

RESULTS: The profiles identified 521 genes whose expression was upregulated in V30M nerves, as compared with those from WT mice, and 223 transcripts downregulated more than 1.5 fold (p<0.05). Distinct signaling pathways were differentially upregulated in the V30M nerve belonging to a diverse set of categories including cell adhesion, developmental processes, regulation of mRNA transcription, extracellular transport and cell communication. In contrast, the pattern of downregulated genes was mostly associated with immunity and defense. From all the upregulated molecules, particular interest relied on Protochaderin10 (Pcdh10) due to the higher fold change (277x).

This data was validated in V30M mice in the naive situation; mRNA and protein analyses displayed overexpression of Pcdh10 by Schwann cells and axons. Prompted by these results, we tested expression of Pcdh10 in human samples. Immunohistochemical analysis of sural nerve biopsies from FAP patients versus asymptomatic V30M carriers and normal control subjects showed evident alterations in Pcdh10 protein levels in advanced stages of disease. Moreover, ELISA for Pcdh10 in plasma samples showed a significant increase in this molecule protein levels with neuropathy progression.

DISCUSSION & CONCLUSIONS: Taken together, these data shed light the important role of Pcdh10 in the pathophysiology of TTR amyloidosis and highlights the potential for this molecule as a novel biomarker for polyneuropathy progression.
Strictly co-isogenic C57BL/6J Prnp<sup>−/−</sup> mice: a rigorous resource for prion science.

M Nuvolone<sup>1*</sup>, M Hermann<sup>1,2*</sup>, S Sorce<sup>1</sup>, G Russo<sup>3</sup>, C Tiberi<sup>1</sup>, P Schwarz<sup>1</sup>, E Minikel<sup>4</sup>, D Sanoudou<sup>5</sup>, P Pelczar<sup>2</sup> and A Aguzzi<sup>1</sup>

<sup>1</sup>Institute of Neuropathology, University Hospital of Zurich, Zurich, Switzerland. <sup>2</sup>Institute of Laboratory Animal Science, University of Zurich, Zurich, Switzerland. <sup>3</sup>Functional Genomics Center Zurich (FGCZ), Zurich, Switzerland. <sup>4</sup>Prion Alliance, Cambridge, MA, USA; Broad Institute, Cambridge, MA, USA; Analytical and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA, USA. <sup>5</sup>Department of Pharmacology, Medical School, University of Athens, Athens, Greece. * Equal contribution

mario.nuvolone@usz.ch

INTRODUCTION:

The cellular prion protein, PrPC, is a ubiquitously expressed membrane-anchored protein encoded by the Prnp gene<sup>1</sup>. Misfolding of PrPC generates the scrapie prion protein PrP<sub>Sc</sub>, and leads to a class of invariably lethal, neurodegenerative conditions termed transmissible spongiform encephalopathies or prion diseases<sup>1</sup>. Recently, PrPC has been shown to bind amyloid β oligomers and mediate their neurotoxic effects and has therefore being suggested as a potential therapeutic target for Alzheimer’s disease<sup>2</sup>. Conversely, the physiologic role of PrPC remains enigmatic. A plethora of functions have been ascribed to PrPC based on phenotypes of Prnp<sup>−/−</sup> mice. However, all currently available Prnp<sup>−/−</sup> lines were generated in embryonic stem cells from the 129 strain of the laboratory mouse and mostly crossed to non-129 strains. Therefore, Prnp-linked loci polymorphic between 129 and the backcrossing strain resulted in systematic genetic confounders and led to erroneous conclusions<sup>3</sup>. In the present study, we aimed at generating a novel Prnp<sup>−/−</sup> mouse line in the well-characterized C57BL/6J background using genome-editing.

MATERIAL & METHODS:

We used transcription activator-like effector nuclease (TALEN)-mediated genome editing in fertilized mouse oocytes to create the Zurich-3 (ZH3) Prnp-ablated allele on a pure C57BL/6J genetic background. To characterize the genome of the Prnp<sup>ZH3/ZH3</sup> mice, we performed spectral karyotyping, array comparative genomic hybridization, Sanger sequencing and targeted analysis of <em>in silico</em> predicted potential off targets. Lack of PrPC was demonstrated by Western blotting, ELISA and immunofluorescence. Transcriptomic analysis was performed using RNA sequencing. Phenotyping of the Prnp<sup>ZH3/ZH3</sup> mice included behavioral, immunologic, histologic and ultrastructural analyses.

RESULTS:

TALEN-genome editing of the Prnp locus resulted in an 8-bp deletion of Prnp exon 3 (termed Prnp<sup>ZH3</sup> allele), leading to a premature stop codon within the PrPC signal peptide, and resulting in the ablation of PrPC when bred to homozygosity. Genomic, transcriptional, and phenotypic characterization of Prnp<sup>ZH3/ZH3</sup> mice failed to identify phenotypes previously described in non-coisogenic Prnp<sup>−/−</sup> mice. However, aged Prnp<sup>ZH3/ZH3</sup> mice developed a chronic demyelinating peripheral neuropathy.

DISCUSSION & CONCLUSIONS:

Our study confirms the crucial involvement of PrPC in peripheral myelin maintenance. This new line represents a rigorous genetic resource for studying the role of PrPC in physiology and disease.

REFERENCES:

Novel mutation in the lysozyme gene leading to hereditary lysozyme amyloidosis with biopsy-proven cardiac involvement

BW Sperry¹, M Hanna¹, A Ikram¹, J Theis², N Leung³, WE Highsmith², M Grogan⁴, A Dispenzieri²,⁵

¹Department of Cardiovascular Medicine, Cleveland Clinic Foundation, Cleveland, Ohio.
²Department of Laboratory Medicine and Pathology, ³Division of Nephrology, ⁴Department of Cardiology, ⁵Division of Hematology, Mayo Clinic, Rochester, Minnesota.

sperryb@ccf.org

ABSTRACT:

We present a case of a patient with a novel mutation in the lysozyme protein leading to familial systemic lysozyme amyloidosis (ALys) with cardiac involvement. A 35-year-old white male initially presented to us with sicca syndrome, uncontrolled hypertension and renal failure. His mother had biopsy-proven amyloidosis affecting the kidney requiring transplantation. The patient was found to have premature hypertension at age 5 and was started on antihypertensive therapy at age 20. He was initially misdiagnosed as AA amyloidosis at age 27 after a duodenal biopsy for the workup of nausea and abdominal pain. He subsequently developed renal dysfunction, progressive volume overload, and diastolic heart failure. The echocardiogram demonstrated increased wall thickness (anteroseptum 14 mm), bialtrial dilation, and a moderate pericardial effusion (Figure 1). Electrocardiogram showed low voltage in the limb and precordial leads (Figure 2). He underwent endomyocardial biopsy which revealed interstitial and vascular amyloid deposition. By mass spectroscopy, the amyloid was found to be due to lysozyme. A liver biopsy did not show cirrhosis but did contain amyloid infiltration as well. Mass spectroscopy of the amyloid was found to be due to lysozyme, with a T88N variant. Genetic testing confirmed T88N and also found the W130R mutation in the lysozyme gene. He died 2 years after diagnosis while being considered for combined heart/kidney transplantation.

Subsequently, his younger brother was also diagnosed with ALys at age 34 with biopsy-proven proteinuric renal and bone marrow involvement. Echocardiography showed a moderate increase in left ventricular wall thickness and a pericardial effusion.

In conclusion, we report a case with a complex allele in the lysozyme gene (T88N/W130R), which manifested as familial amyloidosis with renal, liver and cardiac involvement with Sicca syndrome. While 7 mutations of the lysozyme gene leading to ALys have been described¹, T54A is the only other mutation which had been shown to affect the heart².

REFERENCES:


PC89

Bortezomib-High Dose Melphalan conditioning used as an alternative regimen for a second ASCT in a patient with relapsed Scleromyxedema

VH Jimenez-Zepeda1, A Chaudhry1, P Duggan1, P Neri1 and NJ Bahlis1

1Department of Medical Oncology and Hematology, TBCC, Calgary, AB, Canada

Victor.Zepeda@albertahealthservices.ca

INTRODUCTION Scleromyxedema is a rare paraneoplastic fibromucinous connective tissue disorder characterized by papular mucinosis, monoclonal gammapathy and extracutaneous involvements. It tends to have a chronic and progressive course, with a high mortality rate from extracutaneous involvements. Recent evidence suggests a potential benefit of ASCT for these patients.

METHODS Here, we report a case of scleromyxedema who underwent a second ASCT for relapsed disease by using Bortezomib and HDM conditioning. In addition, MRD assessment of clonal plasma cells was performed by using multiparameter flow cytometry.

RESULTS A 63-years/old male presented with clinically relapsed scleromyxedema in the fall of 2014. Patient was initially treated back in 2002 when He presented with a clinical course of life-threatening scleromyxedema. At that time, oral alkylating therapy did not induce significant response and patient consented to pursue treatment with high-dose melphalan-BU conditioning followed by Autologous Stem Cell Transplantation, obtaining a complete response with an extraordinary clinical remission. Twelve-years later, patient presented again with progression of pruritic ‘hardened shiny lumps’ over diffusely thickened skin. On physical examination, he had shiny firm white papules, particularly over his extremities, and a leonine face with lumps in the glabella that had formed a cobblestone appearance. His face was erythematous and his trunk was hyperpigmented. He also developed significant decreased oral aperture and had severe sclerodactyly. Laboratory investigation revealed a monoclonal protein of 3 g/L consistent with IgG lambda on IFE, albumin of 38 g/l, β-2-microglobulin of 2.71 Mg/l, UPEP revealed 0.17 g/day of proteinuria and IFE in urine was negative. FLC assay revealed a free kappa of 21.6, lambda 11.9 and k/l ratio of 1.82. Bone marrow aspirate and biopsy showed the presence of 3-5% of monoclonal plasma cells. Pulmonary function tests revealed mild reduction in diffusing capacity. Patient was mobilized again with G-CSF and plerixafor and later on went onto receive a second ASCT with Bortezomib-HDM conditioning. Transplant was well tolerated and engrafted at day-14 with no significant complications. At day-100 post-ASCT patient was confirmed to have complete response with MRD studies in the bone marrow negative for monoclonal plasma cells. Furthermore, significant clinical improvement was observed at 2-months post-ASCT. Patient remains in complete response at 15 months from his second ASCT.

IN CONCLUSION, the current case report supports to the existing literature reporting consistent clinical benefit in using high-dose chemotherapy and ASCT for scleromyxedema. The present case exhibits the feasibility of salvage ASCT for patients whose first response to initial ASCT was durable. It also suggests that bortezomib and melphalan conditioning is feasible and efficacious in this setting. Based on the above mentioned, further validation of this conditioning regimen is required.

REFERENCES


Implementation of next generation sequencing (NGS) in samples with suspected hereditary amyloidosis – first experience

Z Kufova1,2,3, J Januska4, T Sevcikova1,2, T Jelinek1, F Kryukov1,2, R Hajek1,2,3

1Department of Hematooncology, University Hospital Ostrava, Ostrava, Czech Republic. 2 Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic. 3 Babak Myeloma Group, Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic. 4 Cardiocentre Podlesi, Trinec, Czech Republic.

z.kufova@gmail.com

INTRODUCTION: Hereditary amyloidosis is a disease caused by deposits of misfolded proteins (amyloids) in various tissues resulting in end organ damage. Formation of hereditary amyloidosis has been associated with germline mutations in 7 genes coding for amyloidogenic precursor proteins. Damage of different organs is linked with specific mutations in certain genes, thus it is possible to reduce the number of genes for examination based on the presented symptoms. At present, Sanger sequencing is routinely used for evaluation of mutations in amyloidogenic genes, however the cost of this approach is relatively high. The more cost effective method that would cover broad range of target genes is still lacking, although employment of modern high throughput sequencing methods has potential to fill this gap. We designed a panel of 11 amyloidogenic genes including 7 common genes involved in hereditary amyloidosis along with 4 genes associated with other “amyloid diseases” (e.g. Alzheimer or prion diseases). By this approach, we are able to examine patients with suspected hereditary amyloidosis or “amyloid diseases” and confirm the diagnosis when positive results are obtained.

MATERIAL & METHODS: Amyloidosis panel was designed using HaloPlex Custom kit (Agilent Technologies, Santa Clara, USA). This panel includes TTR, FGA, APOA1, APOA2, LYZ, GSN, CST3 together with 4 genes PRNP, APP, B2M, ITM2 associated with “amyloid diseases”. The set of capture probes was created to cover entire gene lengths (all exons and introns) spanning finally 70 kb. Blinded cohort of 43 patients was analyzed together with 4 positive and 4 negative control samples validated previously using Sanger sequencing. Next-generation sequencing was performed using MiSeq Reagent Kit V2 - 500 cycles (Illumina, Inc., San Diego, CA), coverage 50x.

RESULTS: Altogether, 14 Gb data was generated for 43 patients. Four positive samples (identified by Sanger sequencing) were confirmed for the presence of mutation in genes involved in hereditary amyloidosis also by Next-Gen sequencing. Our preliminary data show repeated findings of various SNPs, predominantly in genes CST3, GSN and PRNP, which are associated with predisposition to amyloid formation. Moreover, in two samples, specific mutation Ala25Thr in CST3 gene was detected. This mutation is associated with higher risk of Alzheimer disease and age-related macular degeneration. This finding raises the intriguing possibility that all amyloid diseases, irrespective of the nature of the amyloid forming protein, share common genomic landscape. Detailed analysis and interpretation of sequencing results will be presented in poster.

DISCUSSION & CONCLUSIONS: We designed and validated target sequencing for 11 genes associated with predisposition to amyloid formation. Enrichment of the set of commonly examined genes with genes linked with other types of amyloidoses improves the diagnostic potential for patients with suspected diagnoses. The full length gene coverage allows us to precisely examine all possible mutations responsible for disease manifestation. Taken together, next-generation sequencing provides higher amount of data for significantly lower price compared to standard sequencing methods. Employment of high throughput techniques is especially beneficial for large cohort analysis.

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“Peptide H”: a highly amyloidogenic two chain fragment of insulin.

W Dzwolak1,2, M Piejko2,3, R Dec1, V Babenko1, A Hoang2,3, M Szewczyk1, P Mak3.

1 Department of Chemistry, University of Warsaw, Warsaw, Poland. 2 Polish Academy of Sciences, Institute of High Pressure Physics, Warsaw, Poland. 3Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland.

wdzwolak@chem.uw.edu.pl

INTRODUCTION: Proteases are recognized for their role in the emergence of highly aggregation-prone protein fragments in vivo. On the other hand, limited proteolysis in vitro is often used to probe different phases of amyloidogenic pathways. Here we show for the first time and explain an “explosive” fibrillation induced by moderate amounts of pepsin in acidified samples of bovine insulin.

MATERIAL & METHODS: Spontaneous fibrillation of insulin from bovine pancreas was studied in the presence of pepsin from porcine gastric mucosa at pH*=1.9 in 0.1 NaCl. Aggregation kinetic rates were probed using Thioflavin T fluorescence and time-lapse FT-IR spectroscopy. Products of partial proteolysis and the composition of fibrils precipitating in the presence of the enzyme were analysed using RP-HPLC, Edman degradation and MALDI TOF spectrometry and CD. AFM was used to study morphology of fibrils.

RESULTS: Figure 1A shows a very significant acceleration of fibrillation taking place in acidified insulin solutions in the presence of small amounts of pepsin, as probed by ThT-fluorescence. Biochemical analysis of the pepsin-induced fibrils reveals previously unreported two-chain peptide with potent amyloidogenic properties as the main building block. The peptide (named ‘H’) comprises of N-terminal fragments of insulin A- and B-chains linked by disulfide bond between Cys7A-Cys7B (Fig. 1B) and conceals up to 8 additional pepsin-cleavage sites which become protected upon fast fibrillation unless concentration of the enzyme is increased leading to complete digestion of insulin [1]. Fibrils built of H-peptides are similar in terms of morphology (as probed by AFM) and infrared features to typical bovine insulin fibrils, but they appear to lack the ability to seed fibrillation of intact insulin. Controlled re-association of these fragments leads to ‘explosive’ fibrillation only under non-reducing conditions implying the key role of the disulfide bonds in the amyloidogenicity of H-peptides.

Fig. 1: Kinetics of amyloidogenesis of insulin in the absence and presence of pepsin (A). Spatial placement of the H fragments (red tubes) within the native structure of bovine insulin dimer (PDB entry 2A3G).

DISCUSSION & CONCLUSIONS: Our study highlights the role of dynamics of the disulfide-bonded N-terminal fragments of A- and B-chains in insulin amyloidogenesis. It also suggests that the core region of insulin amyloid may be different from the one proposed by Ivanova et al. [2]

REFERENCES:


Insulin: A diagnostic warning

JJ Liepniesks, MD Benson

Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, Indiana USA

mdbenson@iupui.edu

INTRODUCTION: While the importance of correctly characterizing the type of amyloid involved with each patient has always been apparent, the advent of new and, hopefully effective, therapies for many of the different forms of amyloidosis has emphasized the role of correct diagnosis. Fortunately, new technologies have become available for biochemical analysis of amyloid tissue biopsies. Immunohistochemistry, amino acid sequencing, and laser dissected mass spec analysis have all enhanced our abilities to correctly type amyloid biopsies biochemically. We present two cases of amyloidosis from human insulin to emphasize the importance of biochemical analysis to avoid misdiagnosis.

MATERIAL & METHODS: A 62-year-old African-American man presented with congestive heart failure and with recurrent pleural effusions. He had a coronary artery bypass without benefit and subsequent cardiac pacemaker with similar lack of therapeutic result. Subsequent echocardiogram demonstrated cardiomegaly with suggestion of amyloidosis. Abdominal fat biopsy demonstrated amyloid and the patient was referred to the Amyloid Center. DNA analysis was negative for the TTR Val122Ile mutation and evaluation for AL amyloidosis was nonrevealing. An endomyocardial biopsy did not show amyloid. Extraction of amyloid from the abdominal fat biopsy and amino acid sequencing demonstrated a sequence consistent with human insulin. Subsequent laser dissected mass spec analysis from the same biopsy confirmed this finding. In retrospect, a many-year history of subcutaneous insulin injection for diabetes mellitus was confirmed for this patient.

A second case is a 24-year-old man with Rabson-Mendenhall syndrome who required as much as 1900 units of insulin daily delivered via two subcutaneous pumps to maintain a functional blood glucose level. This gentleman presented with painful masses in the groin which, on biopsy, proved to be lymph nodes infiltrated with amyloid. He also had axillary masses which were of lesser size. Amyloid extraction from paraffin embedded groin biopsies followed by amino acid sequencing revealed a sequence compatible with human insulin.

DISCUSSION & CONCLUSIONS: Abdominal fat aspiration or biopsy has become a mainstay in diagnosis of systemic amyloidosis and, while it has greater sensitivity with AL than with hereditary types of amyloidosis, it has greatly facilitated timely diagnosis and treatment. It cannot be assumed, however, that amyloid demonstration on abdominal fat biopsy or regional lymph node biopsy is indicative of systemic amyloidosis as is illustrated by the two cases presented here. The importance of biochemical typing of amyloid remains paramount.
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Fibrinogen A alpha-chain amyloidosis: a non-negligible cause of chronic kidney disease in dialysis patients

†Tavares1,2, L Moreira3, PP Costa3,4, L Lobato4,5

1Department of Nephrology, Centro Hospitalar de São João, Porto, Portugal. 2Nephrology and Infectious Diseases Research and Development Group, INEB (I3S), University of Porto, Porto, Portugal. 3Department of Human Genetics, National Health Institute Doutor Ricardo Jorge, Porto, Portugal. 4Unit for Multidisciplinary Research in Biomedicine, Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Porto, Portugal. 5Department of Nephrology, Centro Hospitalar do Porto, Hospital de Santo António, Porto, Portugal.

isabel.salome@hsjoao.min-saude.pt

INTRODUCTION: Fibrinogen A alpha-chain amyloidosis (AFib) is a rare, late-onset, autosomal dominant disorder, which presents with chronic kidney disease (CKD). A patient with AFib, homozygous for the FGA p.Glu545Val mutation, was identified in the district of Braga, Northern Portugal (1). Afterwards, we designed a study to assess the prevalence of AFibE526V (p.Glu545Val) among Portuguese patients undergoing dialysis in the same geographical area, through genetic testing for the FGA p.Glu545Val mutation.

MATERIAL & METHODS: A total of 267 prevalent hemodialysis patients, treated at 3 dialysis centres across Braga district, were evaluated between 2005 and 2010. Genetic testing for the FGA p.Glu545Val mutation was offered to patients with CKD attributable to (i) unclassified amyloidosis; (ii) unknown etiology; (iii) glomerular and interstitial diseases, diabetic nephropathy without retinopathy and hypertensive nephrosclerosis, in the absence of histological confirmation. Overall, 122 hemodialysis patients (aged 65.1±14.8 years, 61.5% males) accepted to participate in this study. In addition, for mutation carriers, tissue samples were retrieved from the archives for histological evaluation and family trees were obtained. Prevalence of the disease was assessed by genotyping results.

RESULTS: Overall, FGA p.Glu545Val mutation was identified in 12 (6 unclassified amyloidosis, 3 hypertensive nephrosclerosis, 3 unknown etiology) of 267 hemodialysis patients and it was the most common amyloidotic nephropathy. The typical natural history of AFib in our cohort was of early onset hypertension (52.6±11.8 years), followed by stage ≥3 of CKD at 59.7±10.8 years, detection of proteinuria at 60.9±11.5 years and onset of hemodialysis at 64.8±11.2 years. Mean age at genetic testing was 67.4±12.3 years. Renal biopsies of 6 patients were retrieved from the archives. Amyloid deposition was abundant in the glomeruli and variable in arterioles and cortical interstitium. The immunohistochemistry study disclosed amyloid deposits that stained with an antibody against fibrinogen. Furthermore, amyloid deposits were identified in surgical samples from spleen (n=1), abdominal fat (n=1), colon and ileum (n=1). The 6 males and 6 females diagnosed with AFibE526V (p.Glu545Val) belonged to 10 unrelated families that were unaware of their condition. Additionally, family trees disclosed 5 deceased relatives who underwent hemodialysis in the same centres, likely to have had an unrecognized AFib.

DISCUSSION & CONCLUSIONS: Our findings, which were based on genotyping analysis, disclosed the diagnosis of AFibE526V (p.Glu545Val) in 12 patients corresponding to a disease prevalence of 4.5% in dialysis patients from the district of Braga. Unrecognized AFib, in a non-negligible number of dialysis patients, resulted from lack of physician’s awareness. A high index of suspicion should be reserved for Portuguese patients, from the district of Braga, with unexplained chronic kidney disease. Genetic testing for FGA p.Glu545Val mutation should be offered in order to detect new cases and improve the prognosis of the disease through early diagnosis and treatment.

REFERENCES

Different populations – different prevalence
Or, what is going on in the European brown hare (*Lepus europaeus*)

A Posautz¹, A Kübber-Heiss¹, P Westermark²

¹ Research Institute of Wildlife Ecology, University of Veterinary Medicine Vienna, Austria  ² Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden.

INTRODUCTION

Systemic amyloidosis has rarely been described in the captive or in the free-ranging European brown hare (*Lepus europaeus*) but since the animals are living with chronic diseases, the existence and the development of AA amyloidosis should be expected. Having been able to dissect several hundreds of hares from different geographical areas in the last years a varying occurrence of the disease in two populations was surprising. Therefore a first closer look at the distribution and the kind of amyloid was performed to pave the way for in depth research.

MATERIAL & METHODS:

In the years 2010 and 2011 a total of 298 hares underwent a thorough pathological screening. 188 hares (125 male, 63 female) originated from Lower Austria (LA) and were shot during an ongoing genetics project. The other 110 hares (48 male, 62 female) were sampled in northern Germany (NG) during the normal hunting season. Pathohistology was performed of all organs. Where needed Congo red staining was done. To further analyse the findings and to identify the amyloid immunohistochemistry (IHC) was performed.

RESULTS:

In total 42 animals of the LA population (22.3%; 26 in 2010, 16 in 2011), and only 2 (3.8%; 0 in 2010, 2 in 2011) from NG showed varying degrees of amyloid deposition. Organs affected were liver, spleen, kidneys and in 2 animals the small intestine. Macroscopically all animals showed varying degrees of hepatosplenomegaly. In pathohistology deposition of a homogenous eosinophilic extracellular material was noted. Amyloid was confirmed using Congo red staining. The IHC of the samples showed AA amyloidosis.

DISCUSSION & CONCLUSIONS:

As mentioned, until now only few cases of amyloidosis in brown hares have been reported in the literature. Therefore it is not only of interest finding such high numbers of amyloid in one population, but also the difference between the two populations is astounding. The overall health status of the two populations did not differ as significantly as to explain the difference. As the disease, and if there is transmission is not well described in wildlife, one can only speculate about why and how these differences appear. Further investigations will show, if different environmental conditions, such as climate, habitat, and proximity to livestock and wild birds play a role in occurrence and development of the disease.

REFERENCES:

nanobody gene therapy in the gelsolin amyloidosis mouse model via adeno-associated virus type 9

Adriaan Verhelle¹, Nisha Nair³, Wouter Van Overbeke¹, Olivier Zwaenepoel¹, Cindy Peleman², Nick Devoogdt², Tony Lahoutte², Thierry VandenDriesche³, Marinee Chuah³ and Jan Gettemans¹

¹Department of Biochemistry, Ghent University, Faculty of Medicine & Health Sciences, Albert Baertsoenkaai 3, B-9000 Ghent, Belgium
Adriaan.verhelle@ugent.be

²In vivo Cellular and Molecular Imaging, Free University of Brussels, Laarbeeklaan 103, B-1090 Brussels, Belgium

³Department of Gene Therapy & Regenerative Medicine, Free University of Brussels, Laarbeeklaan 103, B-1090 Brussels, Belgium

INTRODUCTION: Gelsolin amyloidosis (AGel) is an autosomal dominant disease caused by a point mutation (G654A/T is most common) in the gelsolin gene. The corresponding amino acid substitution result in the loss of a Ca²⁺ binding site in the second domain. Subsequent incorrect folding exposes an otherwise internally buried furin cleavage site. During trans-Golgi transport, mutant plasma gelsolin (PG*) gets cleaved by furin leading to the formation of a 68 kDa C terminal fragment (C68). On its turn C68 gets cleaved by membrane type 1-matrix metalloproteinase (MT1-MMP), during secretion in the extracellular matrix, thereby releasing 8 and 5 kDa amyloidogenic peptides.

Single-domain antibodies or Nanobodies were raised against both PG* and the 8 kDa peptide. Both in an in vitro and in vivo setup, one nanobody (Nb11) was able to inhibit furin cleavage and three other nanobodies (FAF Nb1-3) could inhibit MT1-MMP. Both approaches led to a significant reduction in amyloidogenic gelsolin buildup [1, 2].

MATERIAL & METHOD: Now, Nb11 and FAF Nb1 have been combined into a single bispecific format, joined via an MT1-MMP sensitive linker. The hypothesis is that a combined strategy will result in a synergetic effect on the reduction of amyloidogenic gelsolin buildup. This has already been confirmed in vitro and is currently being tested in vivo.

For the in vivo test phase of the bispecific Nb11-FAF1 nanobody, adeno-associated virus type 9 (AAV type 9) gene therapy was used. Neonatal gelsolin amyloidosis mice were injected with 4.7x10¹⁰ vg/mouse [3].

RESULTS: At the age of 3 months the animals will be analyzed using SPECT/CT, immunohistochemistry and ex vivo muscle performance.

Caloric restriction prevents the progression of murine AApooAII amyloidosis.

J Sawashita¹,², L Li², Y Liu², X Ding², M Yang², Z Xu², K Higuchi¹,²

¹Department of Biological Sciences for Intractable Neurological Diseases, IBS-ICCER, Shinshu University, Matsumoto, Japan. ²Department of Aging Biology, Institute of Pathogenesis and Disease Prevention, Shinshu University Graduate School of Medicine, Matsumoto, Japan.

INTRODUCTION: In murine senile amyloidosis, misfolded plasma apolipoprotein (Apo) A-II is deposited as AApooAII amyloid fibrils in a process associated with aging [1]. Nutritional control may be the most plausible and fundamental treatment, in addition to its health promoting capabilities; in fact, caloric restriction (CR) has often been reported as the most effective non-genetic treatment to decelerate aging and extend lifespan and healthspan [2,3]. We demonstrated that CR treatment prevented the progression of AApooAII amyloidosis in mice and investigated the molecular mechanisms.

MATERIAL & METHODS: We used female mice from the R1.P1-Apoa2c congenic strain, which were developed in our laboratory and are prone to AApooAII amyloidosis induction [1]. AApooAII fibrils were obtained from the liver of a mouse showing severe AApooAII deposition [1,4]. Eight-week old mice were assigned randomly into the ad libitum feeding (AL) or CR group. The food intake of the CR group was adjusted weekly according to the intake of the AL group over the preceding week. The CR treatment was performed using a step-down protocol. CR mice were supplied with food at 90% of the food intake weight of the AL group at 8 weeks of age, at 75% at 9 weeks of age, and then at 60% for the following 16 weeks. At 10 weeks of age, i.e., at the first day of switching to 60% CR, half of the mice in the AL and CR groups were injected intravenously with a single dose of 1 µg AApooAII fibrils to induce AApooAII amyloidosis (AL+F and CR+F groups, respectively), and the other mice were injected intravenously with an equal volume of vehicle (PBS) as the controls (AL+V and CR+V groups, respectively). After 16 weeks of amyloidosis induction and 60% CR, all mice were sacrificed by cardiac puncture under deep anesthesia. Plasma and several organs were collected and used for physiological, biochemical, and molecular biological analyses.

RESULTS: The body weights of the CR groups (CR+F and CR+V) did not change or decreased during the intervention, regardless of amyloidosis induction, while those of the AL groups (AL+F and AL+V) increased. In the CR+F group, there were significantly fewer AApooAII amyloid deposits in the liver, skin, tongue and small intestines compared with those in the AL+F group. In the CR groups, fasting blood glucose levels and the areas under the receiver operating characteristic curve according to the intraperitoneal glucose tolerance test at 3 days before sacrifice were significantly less than those before the intervention. Plasma total and HDL-cholesterol levels did not differ among any of the groups after the intervention, but the plasma triglyceride level in the CR+F group was lower than that in the AL+F group. Plasma ApoA-I levels did not differ among any of the groups, but the ApoA-II/ApoA-I ratios were significantly decreased by CR treatment and were further decreased by amyloid induction. In contrast, the mRNA levels of ApoA-II in the liver were not different among those groups. We also observed repressed mRNA levels of inflammation-related factors (NF-κB, TNF-α, IL-1β, IL-6, and TGF-1β) and oxidative stress-associated markers (p47phox and p67phox) in the CR groups. Moreover, CR treatment induced activation of PGC-1α, a major factor that controls mitochondrial biogenesis and respiration, as well as SIRT1.

DISCUSSION & CONCLUSIONS: CR treatment prevented the progression of murine AApooAII amyloidosis. We need more investigations into the molecular preventive mechanisms of CR treatment for amyloidosis, but we believe that CR treatment plays a role in the maintenance of certain metabolic processes, e.g., glucose tolerance, inflammation, mitochondrial function, and especially ApoA-II in the circulation.

A novel form of systemic amyloidosis

I Bouteau¹, ², M Colombat³, L Ecottièrê¹, ², JM Goujon⁴, C Debiais⁴, D Joly⁵, MC Machet⁶, C Barbet⁷, F Bridoux¹, ²

¹ Department of Nephrology, Dialysis and Renal Transplantation, University Hospital of Poitiers, France. ² Centre de référence Amylose AL et autres maladies par dépôts d’immunoglobulines monoclonales, Poitiers, France. ³ Department of Pathology, University Hospital of Toulouse, Toulouse, France. ⁴ Department of Pathology, University Hospital of Poitiers, Poitiers, France. ⁵ Department of Nephrology, Necker Hospital, Paris, France. ⁶ Department of Pathology, University Hospital of Tours, Tours, France. ⁷ Department of Nephrology, University Hospital of Tours, Paris, France.

i.bouteau@laposte.net

Amyloidosis is a group of diseases characterized by extracellular deposition of insoluble fibrils from misfolded proteins. Renal involvement is common in systemic forms. Approximately thirty precursors have been identified, among which ten involved in renal amyloidosis. Some cases of renal amyloidosis still remain unclassified.

We describe a 48-year-old female who presented with persistent proteinuria. She had a history of type 2 multiple endocrine neoplasia, and was discovered a medullary thyroid carcinoma thirteen years ago. Despite surgical treatment, several metastasis occurred (bone, bone marrow, uterus, liver, lung). She was noted to have very high blood concentrations of calcitonin since diagnosis. Physical examination revealed normal blood pressure, peripheral edema and microscopic hematuria. Laboratory tests showed glomerular proteinuria without renal failure (serum creatinine 0.63 mg/dL) and without marked inflammatory syndrome (CRP < 1 mg/L). There was no argument for an extra renal involvement. A first kidney biopsy revealed mesangial deposits which stained positive for Congo red with green birefringence under polarized light. Immunofluorescence staining for all known renal amyloid fibril proteins were negative, including SAA and immunoglobulin light chains. Over the four following years, she progressively developed nephrotic syndrome with renal failure (serum creatinine 1.5 mg/dL). A second kidney biopsy showed predominant glomerular amyloid deposits. She finally died from her cancer with undiagnosed amyloidosis. The kidney biopsy blocks were subsequently reviewed for amyloid typing. Immunohistochemistry staining for calcitonin was strongly positive on glomerular amyloid deposits. Immunoelectron microscopic analysis showed that the renal amyloid fibrils were specifically labelled by gold-conjugated anti-calcitonin antibody. Amyloid subtyping was finally confirmed by laser microdissection/mass spectrometry (LMD/MS)-based proteomics on the first kidney biopsy.

Calcitonin amyloidosis is very common within tumor tissue of medullary thyroid carcinoma but it has never been described as a systemic disease. The structural characteristics of calcitonin, the high blood concentrations, the long time exposure and the preferential interaction of calcitonin with negatively charged phospholipids may explain widespread deposition of calcitonin amyloidosis. Up to our knowledge, we describe the first calcitonin systemic amyloidosis.
Probing medin monomer structure and its amyloid nucleation using rapid $^{13}$C-detected NMR in combination with structural bioinformatics

HA Davies$^1$, DJ Rigden$^1$, MM Phelan$^{12}$ and J Madine$^1$

$^1$ Department of Biochemistry, University of Liverpool, Liverpool, U.K.  $^2$ Technology directorate  J.Madine@liv.ac.uk

INTRODUCTION: Aortic medial amyloid (AMA) is the most prevalent amyloid disease discovered to date, however there is remarkably little known about this disease. AMA is characterized by aberrant deposition of a 5.4 kDa protein called medin within the medial layer of large arteries$^1$. Amyloid proteins are notoriously difficult to study in their soluble form due to their transient and heterogeneous nature, but these early stages provide key information and opportunities for therapeutic targeting. Here we demonstrate a combined experimental and computational approach to elucidate the early stages of medin nucleation.

MATERIAL & METHODS: Ab initio modelling was performed using Quark$^2$ and a locally run version of Rosetta$^3$. $^{13}$C-detected solution NMR was carried out on $^{13}$C/$^{15}$N uniformly labelled medin, on Bruker spectrometers operating at 800 and 600 MHz fitted with TXO and TCI probes respectively.

RESULTS: Together, ab initio modeling and $^{13}$C-detected solution NMR were able to generate a model for soluble monomeric medin comprising a stable core of three $\beta$-strands and shorter more labile strands at the termini. Subsequent molecular dynamics measurements suggested that detachment of the short, C-terminal $\beta$-strand from the soluble fold exposes key amyloidogenic regions allowing for dimerisation and subsequent fibril formation.

DISCUSSION & CONCLUSIONS: This information is critical for understanding the initiation and progression of AMA and enhances our understanding of protein aggregation in general. Furthermore, it provides opportunities to design compounds to modulate medin self-association.


Fig. 1: Proposed mechanism of medin nucleation. Movement of the C-terminal strand increases amyloid propensity (blue) and provides a large hydrophobic face for dimer formation.
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Renal involvement of systemic calcitonin amyloidosis in a patient with medullary thyroid carcinoma

T Koopman¹, C Niedlich - den Herder¹, CA Stegeman², TP Links³, BPC Hazenberg⁴, A Diepstra¹

¹Department of Pathology; ²Department of Internal Medicine, Division of Nephrology; ³Department of Internal Medicine, Division of Endocrinology; ⁴Department of Rheumatology & Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands.

t.koopman@umcg.nl, b.p.c.hazenberg@umcg.nl

CASE REPORT

A 45-year-old woman presented with medullary thyroid carcinoma with diffuse bone metastases, treated with thyroidectomy. During follow-up, clinical progression was slow but the serum calcitonin levels were rising from 150,000 to 400,000 ng/L [normal 0.3 – 12 ng/L]. After four years, tumor marker progression accelerated to a serum calcitonin of 1,076,000 ng/L. Systemic therapy with a tyrosine kinase inhibitor (vandetanib) was started. Subsequently, a nephrotic syndrome with slowly progressive renal insufficiency was discovered. As malignancy associated membranous nephropathy or tyrosine kinase inhibitor-induced focal segmental glomerulosclerosis was suspected, a renal biopsy was performed. Surprisingly, the biopsy revealed diffuse glomerular deposition of calcitonin amyloid (ACal), which was also present in a subsequently taken abdominal fat pad biopsy. Although localized calcitonin amyloid is a characteristic feature of medullary thyroid carcinoma, the systemic calcitonin amyloidosis presented in this case report has not previously been described and may be the result of longstanding extremely high levels of serum calcitonin, facilitating systemic ACal amyloid deposition. Our case illustrates that systemic ACal amyloidosis should be considered in the differential diagnosis of proteinuria in patients with medullary thyroid carcinoma.

Fig 1: Images of a glomerulus in the kidney biopsy of this patient, 400x magnification. Amorphous glomerular amyloid is visible in Hematoxylin-Eosin (A), Periodic Acid-Schiff (B) and Jones’ Silver (C). The amyloid stains Congo Red positive (D) with apple-green birefringence under polarized light (E). Immunohistochemistry shows strong positivity with calcitonin (F) and is negative with amyloid AA (G) and transthyretin (H). In immunofluorescence, there is no predominant staining of kappa (I) or lambda (J) light chains. Abdominal fat pad biopsy revealed Congo Red positive amyloid in subcutaneous fat tissue (K & L).
An Asp to Asn mutation is a toxic trigger in Beta-2 microglobulin: structure and biophysics

Levon Halabelian¹, Matteo de Rosa¹, Alberto Barbiroli², Carlo Santambrogio³, Tanguy Le-Marchand⁴, Rita Grandori³, Guido Pintacuda⁴, Martino Bolognesi¹ Carlo Camilloni⁶, Vittorio Bellotti⁵, Stefano Ricagno¹

¹ Dept. of Bioscience, Univ. of Milan (Italy); ² Dept. DeFENS, Univ. of Milan (Italy); ³ Dept. of Biotech and Biosc. Univ. of Milan Bicocca (Italy); ⁴ CRMN, CNRS/ENS Lyon/UCB Lyon 1 Lyon France; ⁵ Center for Amyloidosis and Acute Phase proteins, UCL, London (UK); ⁶ Dept. of Chemistry, University of Cambridge (UK).

stefano.ricagno@unimi.it

INTRODUCTION:

Beta-2 microglobulin (b2m) is part of the Major Histocompatibility Complex (MHC) and as monomer becomes an aggregation prone protein that is responsible for a human disorder known as dialysis related amyloidosis. In 2012 a new systemic familial amyloidosis was reported: an unreported b2m mutant (D76N) is the etiological agent of such disease. In vitro from the biophysical point of view the D76N b2m is much less stable and more amyloidogenic than wt b2m; however, its crystal structure reveals few minor conformational changes compared with the wt protein (1).

RESULTS:

The Unfolded Protein Response in the ER should target such an unstable protein for degradation, however this is not the case. In order to understand what is the role of the MHC complex in the protection of D76N variant against degradation, the biophysical and structural aspects of MHC complex bearing the D76N mutation have been investigated and evaluated structurally and biophysically (2).

In order to understand the chemical bases of the D76N instability and aggregation propensity two sets of single b2m mutants have been prepared. First all Asp in b2m sequence have been mutated to Asn and the biophysical properties of the mutants have been evaluated indicating that D to N mutation in any position other than 76 does not trigger relevant effects (3).

Therefore a broader study of the molecular bases of D76N peculiar properties is being carried out including in molecular dynamic simulations, molecular dynamics measured by solid state NMR and the biophysical characterisation of mutants at position 76 (D76A, D76H, D76E, D76K).


Cellular interaction and cytotoxicity of amyloid fibrils of the Iowa mutant of apolipoprotein A-I (apoA-I\textsubscript{Iowa}) are mediated by sulfate moieties of heparan sulfate

Kazuchika Nishitsuji\textsuperscript{1}, Kaori Kuwabara\textsuperscript{2}, Kenji Uchimura\textsuperscript{3}, Shang-Cheng Hung\textsuperscript{4}, Norihiro Kobayashi\textsuperscript{5}, Hiroyuki Saito\textsuperscript{6}, Naomi Sakashita\textsuperscript{1}

\textsuperscript{1} Department of Molecular Pathology, Institute of Biomedical Sciences, Tokushima University Graduate School; \textsuperscript{2} Department of Molecular Physical Pharmaceutics Tokushima University Graduate School; \textsuperscript{3} Department of Biochemistry, Nagoya University Graduate School of Medicine; \textsuperscript{4} Genomics Research Center, Academia Sinica; \textsuperscript{5} Department of Bioanalytical Chemistry, Kobe Pharmaceutical University; \textsuperscript{6} Department of Biophysical Chemistry, Kyoto Pharmaceutical University

nishitsuji@tokushima-u.ac.jp

INTRODUCTION: The single amino acid mutation G26R in human apolipoprotein A-I (apoA-I) is associated with AApoAI amyloidosis, and apoA-I carrying this mutation (apoA-I\textsubscript{Iowa}) forms amyloid fibrils in vitro. Heparan sulfate (HS) is a glycosaminoglycan (GAG) that is abundant at the cell surface and in the extracellular matrix. Although HS and its highly sulfated domains are involved in aggregation of various amyloidogenic proteins, the role of HS in AApoAI amyloidosis has never been addressed.

MATERIAL & METHODS: The G26R apoA-I (1-83) fragment was expressed in \textit{Escherichia coli}, purified, and incubated for 7 days at 37 °C to obtain apoA-I\textsubscript{Iowa} fibrils. To assess the role of cellular HS in the interaction between apoA-I\textsubscript{Iowa} fibrils and mammalian cells, we used CHO-K1 cells, pgsD-677 cells which is an HS-deficient variant, and CHO-K1 cells that stably express the human extracellular endoglucosamine 6-sulfatases HSulf-1 and HSulf-2 (CHO-HSulf-1 and CHO-HSulf-2). Cellular interaction of apoA-I\textsubscript{Iowa} fibrils was analyzed by dot blotting and immunocytochemistry by using an anti-apoA-I antibody. Cytotoxicity of apoA-I\textsubscript{Iowa} fibrils was assessed by the MTT assay. β-xyloside was used to eliminate cell surface HS, and sulfation of HS was inhibited by using sodium chlorate. Interaction between heparin and apoA-I\textsubscript{Iowa} fibrils was analyzed by a nitrocellulose filter assay and an ELISA.

RESULTS: Wild-type CHO cells, but not pgsD-677 cells, demonstrated interaction of apoA-I\textsubscript{Iowa} fibrils after incubation with the fibrils. Addition of sulfated GAGs to culture media prevented cellular interaction and cytotoxicity of apoA-I\textsubscript{Iowa} fibrils. Elimination of cell surface HS or inhibition of HS sulfation with chemical reagents interfered with interaction of apoA-I\textsubscript{Iowa} fibrils with CHO cells. We also found that cellular interaction and cytotoxicity of apoA-I\textsubscript{Iowa} fibrils were significantly attenuated in CHO-HSulf-1 and CHO-HSulf-2 cells.

DISCUSSION & CONCLUSIONS: Our results suggest that cell surface HS mediates cellular interaction and cytotoxicity of apoA-I\textsubscript{Iowa} fibrils and that enzymatic remodeling of HS mitigates the cytotoxicity.

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Diagnosis, pathogenesis and outcome in leucocyte chemotactic factor 2 amyloidosis (ALECT 2)

T Rezk, JA Gilbertson, D Rowczenio, HJ Lachmann, AD Wechalekar, M Fontana, S Mahmood, S Sachchithanantham, CJ Whelan, PP Mangione, N Rendell, GW Taylor, PN Hawkins, JD Gillmore

National Amyloidosis Centre, Centre for Amyloidosis and Acute Phase Proteins, Division of Medicine, Royal Free Campus, University College London, UK.

t.rezk@ucl.ac.uk

Introduction: Leucocyte chemotactic factor 2 (ALECT2) amyloidosis was first identified in 2008(1) and data from the US suggests that it is the third most common type of renal amyloid(2) and second commonest cause of hepatic amyloidosis(3). We report the first European case series of the prevalence, clinical presentation, diagnostic findings and clinical course of ALECT2 amyloidosis.

Methods: Results of diagnostic and serial assessments from all patients with ALECT2 amyloidosis who were prospectively followed at the National Amyloidosis Centre (NAC) between 1994 and 2014 are reported.

Results: Twenty-four non-Caucasian patients with ALECT2 amyloidosis comprised 1.3% of those referred to the NAC with biopsy proven renal amyloid. Diagnosis, at median age 62 years, was usually from renal histology. No patient had evidence of cardiac amyloidosis or amyloid neuropathy. Median eGFR at diagnosis was 33 ml/min and median proteinuria was 0.5 g/24 hr. Hepatic amyloid, evident on SAP scintigraphy in 11/24 cases, did not cause significant derangement of liver function. Median follow up was 4.8 years (range 0.5–15.2). Four patients died and 4 progressed to ESRD. Median estimated patient survival was 15.1 years from diagnosis (Fig 1). Mean rate of GFR loss was 4.2 ml/min/yr (range 0.5-9.6) and median estimated renal survival from diagnosis was 8.2 years (Fig 2). Serial SAP scans revealed little or no change in total body amyloid burden.

Conclusions: ALECT2 amyloidosis is a newly identified, relatively benign type of renal amyloid in non-Caucasians which is typically associated with low level proteinuria and a slow GFR decline. It can be reliably diagnosed by immunohistochemistry. Given the discrepancy between the clinical and histological prevalence, it may be much underdiagnosed.

The first Ostertag type amyloidosis in Japan: a sporadic case of hereditary fibrinogen amyloidosis associated with a novel frameshift variant

M Yazaki¹,2, T Yoshinaga¹, Y Sekijima¹,2, F Kametani³, S Nishio³, Y Kanizawa³, S Ikeda¹,2

¹Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan.
²Institute for Biomedical Sciences, Shinshu University, Matsumoto, Japan.
³Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan.
⁴Division of Rheumatology, Endocrinology and Nephrology, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan.
⁵Department of Hematology, Oji General Hospital, Tomakomai, Hokkaido, Japan

mayazaki@shinshu-u.ac.jp

INTRODUCTION

Large numbers of patients with hereditary non-neuropathic and systemic amyloidosis, (Ostertag type amyloidosis), have been described mainly in Western countries, but this type of amyloidosis is quite rare in Asia. In particular, no cases have yet been reported in Japan. Here, we report a patient with fibrinogen amyloidosis associated with a novel variant of fibrinogen Aα-chain gene.

MATERIAL & METHODS

The patient was a 40-year-old Japanese woman presenting with rapidly progressive nephropathy starting at age 32. Renal biopsy demonstrated heavy amyloid deposits mainly on glomeruli. She had long been diagnosed with primary AL amyloidosis due to no family history. Her renal function rapidly deteriorated despite the treatments with chemotherapy and hemodialysis was started at 33 years. At age of 40 years, she was referred to another hospital for further examination. To identify the type of renal amyloidosis, we re-investigated a formalin-fixed paraffin embedded renal tissue sample obtained at age 32 using a laser microdissection (LMD) system.

RESULTS

Proteomics analysis of the glomerular amyloid using an LMD-liquid chromatography-tandem mass spectrometry (LC-MS/MS) revealed that amyloid fibril protein was composed of fragment of the carboxyl terminal portion of fibrinogen Aα-chain. DNA analysis showed a novel heterozygous frame-shift mutation (4-bp deletion) in the fibrinogen Aα-chain gene (c.4899_4902delAGTG).

DISCUSSION & CONCLUSIONS

This is the first case of hereditary fibrinogen Aα-chain amyloidosis in Japan. Even in Japan, patients with Ostertag type amyloidosis are present and the majority of them may have been misdiagnosed with other types of amyloidosis, such as AL amyloidosis. Proteomics analysis of deposited amyloid using LMD-LC-MS/MS was of great use for determining the type of amyloidosis in our patient.
Rapid and sensitive amyloid classification using amyloid-type specific antibodies

RP Linke¹, A Meinel¹, JP Chalcroft²

¹ Reference Centre of Amyloid Diseases, Am Klopferspitz 19, Martinsried, D-82152, Germany,
² Max Planck Institute of Neurobiology, Am Klopferspitz 18, Martinsried, D-82152, Germany.

INTRODUCTION:
Amyloidosis represents a large group of fatal diseases caused by different amyloids, in particular when they are systemic. Therefore, all amyloids must be identified on biopsies at the earliest opportunity, followed by typing of their chemical nature for therapeutic considerations.

MATERIAL AND METHODS:
Formalin-fixed tissues were stained using Congo red according to Puchtler et al.(1) and classified immunohistochemically (IHC, double staining CRIC) using amyloid-type specific antibodies against 16 different amyloid classes (2) and in some cases compared with mass spectrometry (MS) in blinded fashion (3). Tissue sections were evaluated microscopically in conventional bright field; in polarized light and by Congo red fluorescence (CRF).

RESULTS:
Correct typing of 16 different amyloids from 728 patients was achieved with 96 % sensitivity and more than 98% specificity. The distribution of the various amyloid classes was published in (1). For detecting the correct amyloid type, both CRIC and CRF were essential. In comparison with MS, IHC showed a significantly better sensitivity and also showed some advantage in specificity (2).

CONCLUSIONS:
Immunohistochemical classification of amyloid is most reliable with amyloid-type specific antibodies and CRIC/CRF. IHC is easy, fast and inexpensive, but it does require expertise.

REFERENCES:
Serum Hevylite assay may assist in differential diagnosis of patients with high suspicion of AL amyloidosis.

D Yogev¹, M Pick¹, E Slyusarevsky¹, G Pogrebijski¹, ME Gatt¹

¹ Department of Hematology, Hadassah Hebrew University Medical Center, Jerusalem, Israel
rmoshg@hadassah.org.il

Introduction: Multiple myeloma (MM) and AL amyloidosis (AL) are two malignant forms of monoclonal gammopathies. Whereas in MM the end organ damage is evident and easily diagnosed, AL is insidious, and its end-organ damage (i.e. mostly nephropathy and cardiomyopathy) mimics that of common diseases (i.e. hypertension, diabetes mellitus), making the diagnosis difficult. Moreover, monoclonal gammopathy of unknown significance (MGUS), is also a very common state, and when combined with such end-organ damage, it is important to diagnose and distinguish the malignant form of AL. Routine serum/urine tests for monoclonal protein are insufficient for differential diagnosis, and invasive procedures, such as tissue aspiration or biopsy, are utilized. However, even then diagnosis may be deferred and patients are referred for investigation based on the pre-test probability and a clinical suspicion of AL. Preliminary studies show that AL patients have immunoglobulin (Ig) immunoparesis changes secondary to immune dysregulation, as established by Hevylite (HLC) tested pair suppression. We hypothesized that these assays patterns may help discriminate between disease (AL) and “normal” (MGUS) states.

Methods: 98 Serum samples of patients with a high clinical suspicion of AL, were assessed for the presence of disease, and prospectively tested for IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and IgMλ concentrations using Hevylite® assays (The Binding Site Group Ltd, UK) in a blinded manner. The results were correlated with the final diagnosis. Immunoparesis was defined as levels of any HLC isotype below the lower limit of their respective reference range (i.e. in g/L: IgGκ<3.84; IgGλ<1.91; IgAκ<0.57; IgAλ<0.44; IgMκ<0.19; IgMλ<0.12).

Results: Of 98 samples, 43 patients had AL, 3 had light chain deposition disease; 52 patients were under investigation for AL, yet their final diagnosis was MGUS (38), smoldering myeloma (SMM) (5), localized AL (7), and other diseases (2). HLC analysis identified immunoparesis of at least one HLC isotype in 39 (84.8%), most (54.3%) with three or more suppressed. Of the patients with no evidence for AL, 16 (30.8%) had immunoparesis. The median number of HLC isotype paresis was 3 for AL/ LCDD as compared with 0 for non-AL samples (p<0.001). Excluding the five SMM patients (where immunoparesis may be part of the underlying disease) - Using a cutoff of one or more HLC isotypes below the lower limit of the normal range, the sensitivity was 84.8% (95%CI 71.1 to 93.7) and specificity 72.3% (95%CI 57.4 to 84.4); with a negative predictive value of 82.9%, and a positive predictive value of 75%.

Conclusion: HLC measurements for immunoparetic uninvolved Ig isotypes patterns, when one or more are suppressed, may aid in the differential diagnosis of AL in high-risk investigated patients, and may assist decision of further work-up for AL diagnosis.
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