Pitfalls in diagnosis of amyloidosis

Ashutosh Wechalekar
Professor of Medicine and Haematology

National Amyloidosis Centre
University College London

Department Of Haematology
University College London Hospitals
Disclosure of Conflict of Interest

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<table>
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<th>Name of for-profit or not-for-profit organization(s)</th>
<th>Description of relationship(s)</th>
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<td>Any direct financial payments including receipt of honoraria</td>
<td>GSK, Janssen, Celgene, Takeda</td>
<td>Honorarium</td>
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<td>Membership on advisory boards or speakers’ bureaus</td>
<td>Prothena</td>
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<tr>
<td>Funded grants or clinical trials</td>
<td>Amgen</td>
<td>Research funding</td>
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<td>Patents on a drug, product or device</td>
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<td>All other investments or relationships that could be seen by</td>
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<td>a reasonable, well-informed participant as having the potential to influence the content of the educational activity</td>
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</table>
Pitfalls in diagnosis:

- Early recognition
- Correct identification of fibril type
- Appropriate use of diagnostic methods

"The doctor isn't in right now. When you hear the beep, please leave your name, number and a short diagnosis."
Amyloidosis is a difficult diagnosis

- Can involve any organ or organ system in any combination
- Presents with “common” symptoms - Physicians don’t “think” of amyloidosis
- There is *no* single diagnostic blood test for amyloidosis

Amount of amyloid deposition determines outcomes

Fontana M et al, Circulation 2015;132:1570-9
Patients with minimal cardiac symptoms have better outcomes.

Survival

NYHA 0-2

NYHA 3-4

Months

Wechalekar – ALChem 2016 - unpublished
Late diagnosis remains a problem – the heart

**Mayo Cardiac Stage**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Patients</th>
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<tbody>
<tr>
<td>1</td>
<td>14%</td>
</tr>
<tr>
<td>2</td>
<td>27%</td>
</tr>
<tr>
<td>3A</td>
<td>42%</td>
</tr>
<tr>
<td>3B</td>
<td>17%</td>
</tr>
</tbody>
</table>
Late diagnosis remains a problem – the kidneys

Dispenzieri, Blood. 2014 Oct 9;124(15):2315-6
What can we do:
Suspect, Suspect, Suspect....Educate Educate, Educate!

- CKD with Albuminuria > 0.5g/day
- Unexplained fatigue, weight loss, gut symptoms
- Bleeding or soft tissue symptoms
- Heart failure with preserved ejection fraction (HFPEF)
- A patient with "LVH" on echocardiogram and normal or low voltage ECG
- A combination of peripheral and autonomic neuropathy

CKD prevalence by age (UK)

This is simple

**Biopsies!**

- **Bone marrow**
  - 53/144 total patients (36.8%) had bone marrow biopsy
  - 12/20 patients (60%) in whom MGRS was considered had **bone marrow biopsy**

- **Kidney**
  - 19/144 total patients (13.1%) had kidney biopsies
  - 6/20 patients (30%) in whom MGRS was considered had kidney biopsy
Help is on the way..... A blood test for AL?

Multiple myeloma gammopathies

Assay to rapidly screen for immunoglobulin light chain glycosylation: a potential path to earlier AL diagnosis for a subset of patients

Sanjay Kumar¹ · David Murray² · Surendra Dasari³ · Paolo Milani⁴ · David Barnidge² · Benjamin Madden⁵ · Taxiarchis Kourelis¹ · Bonnie Arendt³ · Giampaolo Merli⁴ · Marina Ramirez-Alvarado⁴ · Angela Dispenzieri¹,²

<table>
<thead>
<tr>
<th>Sample type</th>
<th>κ Clone</th>
<th>λ Clone</th>
<th>All patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL, n/N (%)</td>
<td>20/61 (32.8)</td>
<td>13/128 (10.2)</td>
<td>33/189 (17.5)</td>
</tr>
<tr>
<td>Non-AL, n/N (%)</td>
<td>3³/81 (3.7)</td>
<td>2⁴/41 (4.9)</td>
<td>5/122 (4.1)</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt; 0.001</td>
<td>NS</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>12.68</td>
<td>2.20</td>
<td>4.95</td>
</tr>
</tbody>
</table>

Median FLC measurement, mg/dL (IQR)

<table>
<thead>
<tr>
<th>Glycosylated (n = 36)</th>
<th>Non-glycosylated (n = 252)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosylated (n = 36)</td>
<td>34.4 (4.1–90.5)</td>
</tr>
<tr>
<td>Non-glycosylated (n = 252)</td>
<td>50.8 (13.9–79)</td>
</tr>
<tr>
<td>p-Value</td>
<td>NS</td>
</tr>
</tbody>
</table>
Finding amyloid deposits
Where to look for amyloid

- Screening biopsy
  - Abdominal fat aspirate (FA)
- Muchtar et al in 612 pts with AL amyloidosis a BM + FA was diagnostic in 89%

1. Abdominal fat aspirates useful only if laboratory set up to test
2. Cannot rule out amyloid deposition if negative

- AL: 181/216 (84%)
- ATTRmt: 51/113 (45%)
- ATTRwt: 42/271 (15%)


Eur Heart J. 2017 Jun Quarta et al Diagnostic sensitivity of abdominal fat aspiration in cardiac amyloidosis.
Congo Red Staining: national problem

Improvement in amyloid staining leading to accurate diagnosis

UK National External Quality Assurance Scheme for Congo Red

*2014 Amyloid Run 89* → 85% pass, **15% fail** (~45 labs)

*2015 Amyloid Run 94* → 90% pass, **10% fail** (~30 labs)

*2016 Amyloid Run 105 → 71% pass, **26% poor and suboptimal**

NEQAS Assessors report for amyloid – remains 13% who “failed to clearly demonstrate amyloid”

**no change from 2013!**
Problem of Congo red interpretation

<table>
<thead>
<tr>
<th>Year</th>
<th>Fat aspirates</th>
<th>Renal</th>
<th>Bone marrow</th>
<th>Cardiac</th>
<th>Rectal</th>
<th>Skin</th>
<th>Other</th>
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<tbody>
<tr>
<td>2015</td>
<td>329</td>
<td>244</td>
<td>317</td>
<td>70</td>
<td>50</td>
<td>58</td>
<td>216</td>
</tr>
<tr>
<td>2016</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2017</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- False positive rate 2015: 24%
- False positive rate 2016: 17.5%
- False positive rate 2017: 24%
- False positive rate 2018: 24%

- False Negative rate 2015: 12%
- False Negative rate 2016: 10%
- False Negative rate 2017: 9%
- False Negative rate 2018: 12%

- Renal/ urology 2015: 19%
- Head and Neck 2015: 3%
- GI 2015: 11%
- BMT 2015: 21%
- Spleen /Lymph node 2015: 1%
- Soft Tissue 2015: 2%
Even on targeted biopsies

MICROSCOPIC DESCRIPTION

The submitted material is a small fragment of unremarkable myocardium in which there is no morphological or histochemical evidence of amyloid deposition. No inflammatory changes are identified. There is no interstitial fibrosis.

The appearances are within normal limits.

SUMMARY

MYOCARDIAL BIOPSY - NORMAL - NO EVIDENCE OFAMYLOID DEPOSITION
Remember to request Congo red staining: The story of Minimal change disease

(a) CR applied
CR equivocal
CR not applied

(b) EM undertaken
EM not undertaken

(a) Congo red staining
(b) Congo red staining
(c) Congo red staining
(d) Congo red staining
The next challenge is typing:
All patients or selected patients
Amyloidosis is not just AL or ATTR

Types of Hereditary Amyloid in 961 patients

- TTR; 724; 76%
- ApoA1; 52; 6%
- Fibalpha; 134; 14%
- CysC;...
- Gel; 20; 2%
- Lyso; 23; 2%
- Insulin; 18; 0%
- Hered; 961; 11%
- Localised; 962; 11%
- AA; 856; 10%
- LECT2; 34; 0%
- wtTTR, 929, 10%
- AL; 5203; 58%
Organ involvement and amyloid type

Amyloidosis

- Renal involvement
  - AA
    - LECT2
    - ApoA1/Afib
- Liver involvement
  - ApoA1
    - Lysozyme
- Heart involvement
  - Transthyretin
    - ApoA1
- Neuropathy
  - Transthyretin
    - ApoA1
- Soft Tissue
  - Nil

AL amyloidosis
What is and is not AL amyloidosis

- M-protein + amyloid deposits on biopsy

Hereditary amyloidosis:
- 10% of NAC cases
- No Family history in ~ 50%
- Variable phenotype
- Incidental MGUS in 20%+
- Difficult immunohistochemistry
- Multiple mutations – often novel

10% misdiagnosis rate

30% in isolated renal Amyloidosis
Changing demographics of amyloidosis

Number of New Cases by Type

Percentage Breakdown

AL  AA  Hered  wtTTR  Localised


0  50  100  150  200  250  300  350  400  450  500

0%  10%  20%  30%  40%  50%  60%  70%
$^{99m}$Tc-DPD in the heart – very sensitive in ATTR (~100%)
$^{99m}$Tc-DPD for cardiac amyloidosis

Cardiac uptake on DPD  ➔ Amyloidosis

No Cardiac uptake on DPD  ➔ No amyloidosis

Cardiac uptake on DPD  ➔ ATTR amyloidosis
Isolated cardiac AL in Elderly on imaging

**FLC/IFE/SPEP**

- **Monoclonal Gammopathy**
  - YES: Endomyocardial biopsy
  - NO: 99mTcDPD/PYP scan

99mTcDPD/PYP scan

- Positive => gr2 ATTR type
- Negative or grade 1: Consider cardiac Bx

Modified from Gillmore et al Circulation 2016
Patients with wtATTR may have abnormal sFLC or IFE

Diagnosis of AA amyloidosis is (usually) simple

Amyloid A (AA) is an extracellular deposited insoluble fibrillar protein, highly resistant to proteolytic degradation. Such deposition is common for a group of disease known as amyloidosis. The antibody is a useful aid for the identification and classification of AA-amyloidosis.

Amyloid in the medulla, staining positive for DAKO Monoclonal amyloid AA immuno.

But the history is unusual as is the strictly medullary pattern without any glomerular involvement and with minimal proteinuria.
Commercial monoclonal antibodies against:
- AA amyloid (1:600)

Commercial polyclonal antibodies against:
- Amyloid P-component (1:5000), Fibrinogen (1:2000)
- Lysozyme (1:3000)
- Transthyretin (1:4000)
- λ-light chain (1:160000)
- κ-light chain (1:160 000)

Noncommercial polyclonal antibodies directed against:
- apolipoprotein AI (1:1000)
- λ-light chain-derived amyloid proteins (AL1, 1:3000)
## Antibody Panel Used at NAC

<table>
<thead>
<tr>
<th>Antibody</th>
<th>raised in</th>
<th>WORKING DILUTION</th>
<th>SOURCE</th>
<th>antigen retrieval</th>
<th>Absorbed by</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-SAP</td>
<td>Rabbit</td>
<td>1:2000 (TBS/BSA/Azide)</td>
<td>In house</td>
<td></td>
<td>SAP</td>
</tr>
<tr>
<td>anti-SAP</td>
<td>Sheep</td>
<td>1:25000 (TBS/BSA/Azide)</td>
<td>In house</td>
<td></td>
<td>SAP</td>
</tr>
<tr>
<td>P component</td>
<td>Rabbit</td>
<td>1:200 (TBS/BSA/Azide)</td>
<td>DAKO</td>
<td></td>
<td>SAP</td>
</tr>
<tr>
<td>AA (REU 86)</td>
<td>cell supernatant</td>
<td>1:500 (TBS/BSA/Azide)</td>
<td>In house</td>
<td></td>
<td>Acute phase human serum</td>
</tr>
<tr>
<td>AA (REU 86.2)</td>
<td>Mouse</td>
<td>1:100 (TBS/BSA/Azide)</td>
<td>Euro diagnostics</td>
<td></td>
<td>Acute phase human serum</td>
</tr>
<tr>
<td>Amyloid A Component</td>
<td>Mouse</td>
<td>1:100 (TBS/BSA/Azide)</td>
<td>DAKO</td>
<td></td>
<td>Acute phase human serum</td>
</tr>
<tr>
<td>Kappa</td>
<td>Rabbit</td>
<td>1:20000 (TBS/BSA/Azide)</td>
<td>DAKO</td>
<td></td>
<td>normal human serum</td>
</tr>
<tr>
<td>Lambda</td>
<td>Rabbit</td>
<td>1:20000 (TBS/BSA/Azide)</td>
<td>DAKO</td>
<td></td>
<td>normal human serum</td>
</tr>
<tr>
<td>PW lambda</td>
<td>cell supernatant</td>
<td>1:100 (TBS/BSA/Azide)</td>
<td>Per Westermark</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Rabbit</td>
<td>1:1000 (TBS/BSA/Azide)</td>
<td>DAKO</td>
<td></td>
<td>pure lysozyme (sigma)</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Rabbit</td>
<td>1:100 (TBS/BSA/Azide)</td>
<td>Biogenex</td>
<td></td>
<td>pure lysozyme (sigma)</td>
</tr>
<tr>
<td>Fibronogen alpha chain</td>
<td>Sheep</td>
<td>1:300 (TBS/BSA/Azide)</td>
<td>Cambiochem</td>
<td></td>
<td>Normal human plasma</td>
</tr>
<tr>
<td>TTR (prealbumin)</td>
<td>Rabbit</td>
<td>1:4’000 (TBS/BSA/Azide)</td>
<td>DAKO</td>
<td>oxidation antigen</td>
<td>pure prealbumin (sigma)</td>
</tr>
<tr>
<td>Beta-2-Microglobulin</td>
<td>Rabbit</td>
<td>1:500 (TBS/BSA/Azide)</td>
<td>DAKO</td>
<td></td>
<td>no absorption done to date</td>
</tr>
<tr>
<td>Beta-2-Microglobulin</td>
<td>Rabbit</td>
<td>tested on Bond</td>
<td>Leica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>apoA1</td>
<td>Goat</td>
<td>1:4000 (TBS/BSA/Azide)</td>
<td>Genzyme Diagnostics</td>
<td></td>
<td>high density lipoprotein</td>
</tr>
<tr>
<td>Insulin</td>
<td>mouse</td>
<td>1:20 (TBS/BSA/Azide)</td>
<td>Novocastra</td>
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</table>

**Reporting:**

Two persons – blinded reporting
Diagnosis of AA amyloidosis is (usually) simple - at NAC

<table>
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<tr>
<th>Gene</th>
<th>ID</th>
<th>Description</th>
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<tr>
<td>APOA4_HUMAN</td>
<td>2079</td>
<td>Apolipoprotein A-IV OS=Homo sapiens GN=APOA4 PE=1 SV=3</td>
</tr>
<tr>
<td>APOE_HUMAN</td>
<td>482</td>
<td>Apolipoprotein E OS=Homo sapiens GN=APOE PE=1 SV=1</td>
</tr>
<tr>
<td>VTNC_HUMAN</td>
<td>289</td>
<td>Vitronectin OS=Homo sapiens GN=VTN PE=1 SV=1</td>
</tr>
<tr>
<td>SAMP_HUMAN</td>
<td>287</td>
<td>Serum amyloid P-component OS=Homo sapiens GN=APCS PE=1 SV=2</td>
</tr>
<tr>
<td>ALBU_HUMAN</td>
<td>159</td>
<td>Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2</td>
</tr>
<tr>
<td>CLUS_HUMAN</td>
<td>83</td>
<td>Clusterin OS=Homo sapiens GN=CLU PE=1 SV=1</td>
</tr>
<tr>
<td>ACTB_HUMAN</td>
<td>61</td>
<td>Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1</td>
</tr>
</tbody>
</table>
A tale of two diseases
Two types of amyloidosis in one patient

kappa

Apo A1
Moving from diagnosis to identification of organ involvement

Tissue diagnosis and typing

Define underlying clone

Biomarkers for prognostic classification

Imaging for disease extent

Tissue biopsy required

Target organ biopsy

Peripheral tissue biopsy (fat, salivary etc)

Typing

Immunohistochemistry

LC mass spectrometry

- Tissue of choice: abdominal fat
- Innocuous, fast, inexpensive: sensitivity 75-80%, specificity 80-100%

Muchtar E et al Blood. 2017 Jan 5;129(1):82-87
Zeing J et al ISA Japan (abstract) 2018
Fernández de Larrea C Blood 2015;125(14):2239-44
Cardiac amyloidosis may be present with “low” cardiac biomarkers

- Current thresholds for definition: NT-proBNP >332 ng/L
- We reviewed all patients with NT-proBNP <332 ng/L

This has crucial implication for goals of treatment
Cardiac MRI in amyloidosis

AL

ATTR

No LGE  Subendocardial  Transmural LGE
Problem of cardiac MRI interpretation

- Typical patient: 40-60 yr old, history of hypertension and renal impairment
Patients with low presenting dFLC should not be missed

- ~20% of all patients have presenting dFLC <50 mg/L

Reference:
Light chains mass spectrometry may help
Conclusions

• Challenges to diagnosis of amyloidosis persist
• Physician education and early suspicion is crucial
• High prevalence of CKD and HfPEf in the “target” population make screening difficult
• Novel blood tests may allow easier detection
• Typing and correctly following published diagnostic algorithms remain key
• Review in referral laboratories and typing is important
• We have recognise overlap between different types and more patients with mixed amyloid types are being recognised
• Remember to the limitation of imaging and blood tests when diagnosing amyloidosis
I asked life, "Why are you so difficult?"
Life smiled and said, "You people never appreciate easy things."