Diagnosis of Amyloidosis

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Outline

Diagnosis of amyloidosis

Fat pad

Other
Amyloidoses – protein folding disorders

protein quality control systems:
intracellular (proteasomes)
extracellular (macrophages)

Increased concentration
Intrinsic instability
Proteolytic cleavage
mutations

precursor protein  misfolded protein  protofibril  mature fibrils
Amyloidoses

α helix

β pleated sheet

Amyloid formation

Conformational shift to β-pleated sheet $2^\circ$ structure

β-pleated sheet conformation confers affinity to Congo red

*common to ALL types of amyloid*

*Diagnosis of amyloid requires biopsy*
Congo red binding sites
Congo red = bright red color:

- synthesized in 1883 by Paul Bottiger, Friedrich Bayer Company, Germany

- textile dye

- patent sold to the AGFA company of Berlin

- AGFA marketed the dye under the name "Congo red"

1884 Berlin West Africa Conference
Diagnosis of amyloidosis

tissue diagnosis:
- biopsy of an affected organ
- “surrogate” site

Routine stain: extracellular “amorphous” deposits, not-specific for amyloid

Need Congo red stain with green birefringence under polarized light [“apple green” birefringence] = diagnostic

Amyloid is fibrillary only by electron microscopy

think – amyloid order Congo red stain
Detection:

Late diagnosis (left); normal glomerulus on right
Systemic amyloidosis
Pathology:
• kidney, cardiac, peripheral nerves, other sites

Early amyloidosis may be inconspicuous by routine stain
Congo red stain to rule out amyloid and not just to confirm suspicion of amyloid based on routine H&E stain
<table>
<thead>
<tr>
<th>Fibril protein</th>
<th>Precursor protein</th>
<th>Systemic &amp;/or loc</th>
<th>Acquired or hereditary</th>
<th>Target organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL, AH</td>
<td>Immunoglobulin light or heavy chain</td>
<td>S, L</td>
<td>A (H)</td>
<td>All, except CNS</td>
</tr>
<tr>
<td>AA</td>
<td>(Apo) Serum Amyloid A</td>
<td>S</td>
<td>A</td>
<td>All, except CNS</td>
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<tr>
<td>ATTR</td>
<td>Transthyretin, wild type variants</td>
<td>S, S</td>
<td>A</td>
<td>Cardiac, PNS, ANS, heart, eye, leptomeninges</td>
</tr>
<tr>
<td>AApAI, AII, C-III</td>
<td>Apolipoprotein AI, All, C-III, wild type (AIV)</td>
<td>S, S</td>
<td>A, H</td>
<td>Heart, liver, kidney, PNS... kidney</td>
</tr>
<tr>
<td>AIV</td>
<td>Apolipoprotein AI, wild type (AIV)</td>
<td>S, S</td>
<td>A</td>
<td>Kidney primarily</td>
</tr>
<tr>
<td>AFib</td>
<td>Fibrinogen α, variants</td>
<td>S</td>
<td>H</td>
<td>Kidney primarily</td>
</tr>
<tr>
<td>ALECT2</td>
<td>Leukocyte chemotactic factor-2</td>
<td>S</td>
<td>A</td>
<td>Kidney primarily, liver</td>
</tr>
<tr>
<td>Aβ2M</td>
<td>β2Microglobulin, wild type variant</td>
<td>L, S</td>
<td>A</td>
<td>Musculoskeletal ANS</td>
</tr>
<tr>
<td>Cerebral: Aβ, ABri, ACys, APrP</td>
<td>Wild Variants, Wild</td>
<td>L</td>
<td>A</td>
<td>CNS</td>
</tr>
<tr>
<td>Endocrine</td>
<td>ACaI (Pro)calcitonin, Islet amyloid polypeptide (Amylin), Atrial natriuretic factor, Prolactin</td>
<td>L</td>
<td>A</td>
<td>Thyroid (C-cell), Islets of Langerhans, atria, pituitary</td>
</tr>
<tr>
<td>Iatrogenic</td>
<td>AIIns (insulin), AEEnf (Enfurvitide)</td>
<td>L</td>
<td>A</td>
<td>Site of injection</td>
</tr>
<tr>
<td>other</td>
<td></td>
<td></td>
<td></td>
<td>Lung, skin, aorta, cornea...</td>
</tr>
</tbody>
</table>
Renal Amyloidoses – protein types and treatments

AL: ~85%
- derived from immunoglobulin light chain
- clonal plasma cells proliferation
- treatment: anti-plasma cell therapies...

Non-AL: ~15%

AA:
- derived from SAA (serum amyloid-associated)
- chronic inflammation, sporadic or familial
- treatment: anti-inflammatory

ALect2: leukocyte chemotactic factor 2
- pathogenesis?
- no specific therapy

hereditary: avoid misdiagnosis as AL!
- derived from various mutant proteins; transthyretin, fibrinogen, other
- liver transplantation
- clinical trials (transthyretin amyloidosis)
- genetic testing
Differential diagnosis of
differential diagnosis of proteinuria/nephrotic syndrome in adults:

1. Focal and Segmental Glomerular Sclerosis/Minimal change disease
2. Membranous nephropathy
3. Diabetes
4. Amyloidosis!!!

Cardiac amyloidosis – heart failure, arrhythmia, long list of differential
Polyneuropathy – sensory and autonomic disturbances, long list of differential
Amyloid deposits are unevenly distributed in tissues

Congo red stain should be examined routinely on these biopsies!
FAT STORY

Schilder (1909): amyloid frequently present in subcutaneous fat tissue in patients with amyloid A (AA) amyloidosis

P. Westermark and Stenkvist B (1971): diagnosis of secondary (AA) generalized amyloidosis by fine needle biopsy of the skin

Libbey, Skinner, Cohen, 1983, high yield of detection (88%) in AL, ATTR
Amyloid detection in fat – AA, AL, ATTR:

Sensitivity highly variable 54-93%
Specificity: 93-100%

Affected organ – best yield
Other options?
ATTR cardiac amyloidosis

Figure 1. Prevalence of amyloid protein deposition among patients with ATTR cardiac amyloidosis for the study population and for patients with FAC and SSA for (A) any type of noncardiac tissue sampling including noncardiac biopsy or abdominal subcutaneous fat aspiration and (B) only abdominal subcutaneous fat aspiration. Positive = positive for amyloid protein deposition, Negative = negative or equivocal for amyloid protein deposition.

Nowell M. Fine, Adelaide M. Arruda-Olson, Angela Dispenzieri, Steven R. Zeldenrust, Morie A. Gertz, Robert A. Kyle, Paul L. Swieciicki, Christopher G. Scott, Martha Grogan

Yield of Noncardiac Biopsy for the Diagnosis of Transthyretin Cardiac Amyloidosis


http://dx.doi.org/10.1016/j.amjcard.2014.02.030
Fat aspiration was the most commonly performed followed by bone marrow biopsy. Other: rectum, kidney, carpal ligament, liver, small intestine, sural nerve.

<table>
<thead>
<tr>
<th>biopsy</th>
<th>all</th>
<th>Familial ATTR</th>
<th>Wild type senile ATTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat aspirate</td>
<td>225/106+ 47%</td>
<td>141/94+ 67%</td>
<td>84/12+ 14%</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>164/60+ 37%</td>
<td>100/41+ 41%</td>
<td>64/19+ 30%</td>
</tr>
<tr>
<td><strong>heart</strong></td>
<td><strong>131/131+ 100%</strong></td>
<td><strong>42/42+ 100%</strong></td>
<td><strong>89/89+ 100%</strong></td>
</tr>
<tr>
<td>Sural nerve</td>
<td>54/45+ 83%</td>
<td>54/45+ 83%</td>
<td>0</td>
</tr>
</tbody>
</table>
Diagnostic sensitivity of abdominal fat aspiration in cardiac amyloidosis

Amyloid detected on Congo red staining in: 84% cardiac AL, 45% mATTR, 15% wtATTR

Quarta et al, European Heart Journal, Vol. 38, Issue 24, 21 June 2017, Pages 1905–1908,
Coelho et al in FAP:
Labial salivary gland: 89%
Abdominal fat: sensitivity 50-70%
Nerve biopsy: 75-90%
Fat aspirate in wild-type (senile) ATTR amyloid cardiomyopathy

Fine et al 2014, 84 patients, sensitivity of 14%

Ikeda et al 2011, sensitivity increased to 73% (8 of 11 patients), deep layer of surgical fat biopsy, patchy distribution

Takashio et al 2012: amyloid in blood vessels of fat AL > ATTR cardiomyopathy (14 patients)
Pathology of Familial amyloidoses:

1. Detection of amyloid in the index patient
   - lack of a family history
   - new mutation
2. Examination of family members/known carriers
3. Staging, organ involvement
Screening?
↑ awareness
Suspicion → 2\textsuperscript{nd} opinion
Questions?

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