Amyloid and amyloidosis

GEMP 1 14th February 2011
Dr Kirstin Coetzee
Dept of Anatomical Pathology
Amyloidosis

- A disorder of protein metabolism characterised by the extracellular deposition of an abnormal protein.
- Amyloid means “starch-like”; from the Greek *amylon*; name introduced by Virchow in 1854 because it reacted with iodine in a similar manner to starch.
- A pathological proteinaceous substance.
  - The name given to a GROUP of (chemically different) glycoproteins and proteins, which, when deposited in tissues, share the following properties:...
Properties of amyloid:

- Beta-pleated sheet configuration
- Fibrillar ultrastructure
- An affinity for certain dyes: Congo red; Sirius red
- Contains a glycoprotein of the pentraxin family (amyloid P protein)
- Resists removal by natural processes.
- Extracellular deposition, often on basement membranes.
- Causes the affected tissue to become hardened and waxy.
Ultrastructure / Physical nature:

- In thin tissue sections and on electron microscopy:
  - Slightly curved, non-branching FIBRILS
  - 7-10nm in diameter, indefinite length
  - Arranged in bundles
  - May appear hollow on cross-section
  - Each fibril, in turn, consists of 2 – 5 FILAMENTS arranged in a twisted ribbon configuration
  - Fibrils are arranged in a β-pleated sheet configuration (best seen on X-ray crystallography and infrared spectroscopy)
95% of the amyloid material consists of fibril proteins; remaining 5% of the P-component and other glycoproteins (proteoglycans and highly sulphated glycosaminoglycans, incl. heparin sulphate).

There are smaller, doughnut-shaped structures also present between the β-pleated sheets.

These are pentagonal and arranged in stacks, measuring +/-10mm in diameter.

Form the P-component.
Figure 6-53 Structure of an amyloid fibril, depicting the beta-pleated sheet structure and binding sites for the Congo red dye, which is used for diagnosis of amyloidosis. (Modified from Glenner GG: Amyloid deposit and amyloidosis. The &beta; fibrilloses. N Engl J Med 52:148, 1980. By permission of The New England Journal of Medicine.)
Chemical nature

- Chemical composition of the fibrils is related to their precursor proteins.
- There are 15 biochemically distinct forms of amyloid proteins identified.
- 3 most common:
  - AL (amyloid light chain): derived from plasma cells and consists of whole / fragmented immunoglobulin light chains.
  - AA (amyloid associated): derived from a circulating precursor, serum amyloid A. Synthesized in the liver; an acute-phase reactant.
  - Aβ amyloid: is found in the cerebral lesions of Alzheimer disease (core of cerebral plaques; deposited in walls of cerebral blood vessels).
Other amyloid precursors include:

- **Transthyretin TTR (prealbumin):** normal serum protein that binds and transport thyroxine and retinol. A mutant form is deposited in a group of genetically determined disorders – familial amyloid polyneuropathies.

- **β2-microglobulin:** the amyloid fibril subunit (Aβ₂m) that complicates the course of patients on long-term haemodialysis.

- **Prion proteins:** aggregate extracellularly in a minority of cases of CNS prion diseases.
Appearance in tissue sections:

- In sections stained routinely with H&E, amyloid appears as a pink, amorphous hyalinized deposit.
- Evokes no inflammatory response. (In some cases foreign body giant cells may be present.)
- Often found within or close to the wall of a blood vessel.
- Exerts its effects through its physical presence, which distorts the normal architecture, causing atrophy and leading to loss of function.
Figure 6-56 Cardiac amyloidosis. The atrophic myocardial fibers are separated by structureless, pink-staining amyloid (arrows).
Mechanisms of disease:

- Pressure effect on parenchymal cells, leading to atrophy and destruction.
- Narrowing of blood vessels, leading to ischaemia.
- Restricted movement of cardiac muscle.
- Deposition in cardiac conduction system can lead to arrhythmias.
- Increased renal glomerular permeability, leading to proteinuria and the nephrotic syndrome.
Figure 6-55 Amyloidosis of the kidney. The glomerular architecture is almost totally obliterated by the massive accumulation of amyloid.
Special stains:

- Congo Red (and Sirius Red): stains amyloid rose-pink / “salmon-pink”; imparts an apple-green birefringence when seen under polarized light (using one fixed and one rotating polarising filter in the light path on either side of the section).
- Pretreatment of sections with potassium permanganate followed by Congo Red distinguishes between AL and AA amyloid: AL amyloid still binds to CR and AA does not.
- Methyl violet: stains amyloid rose pink and other tissues violet (metachromatic reaction).
- Thioflavine T: fluorescent dye with affinity for amyloid.
- Iodine: stains amyloid (grossly and microscopically) mahogany brown. Addition of 1% sulphuric acid then turns it dark blue.
- Immunohistochemical stains can also distinguish AL from AA amyloid.
Figure 6-52 Amyloidosis. A, A section of the liver stained with Congo red reveals pink-red deposits of amyloid in the walls of blood vessels and along sinusoids. B, Note the yellow-green birefringence of the deposits when observed by polarizing microscope. (Courtesy of Dr. Trace Worrell and Sandy Hinton, Department of Pathology, University of Texas Southwestern Medical School, Dallas TX.)
Figure 6-52 Amyloidosis. A, A section of the liver stained with Congo red reveals pink-red deposits of amyloid in the walls of blood vessels and along sinusoids. B, Note the yellow-green birefringence of the deposits when observed by polarizing microscope. (Courtesy of Dr. Trace Worrell and Sandy Hinton, Department of Pathology, University of Texas Southwestern Medical School, Dallas TX.)
Pathogenesis

- Results from abnormal folding of proteins, which are deposited as fibrils in extracellular tissues and disrupt normal function.
- Diverse range of conditions results in excessive production of proteins that are prone to misfolding.
  - Normal proteins that have an inherent tendency to fold improperly, produced in increased amounts
  - Mutant proteins that are structurally unstable and prone to misfolding and aggregation.
- Increased amounts of these proteins not invariably associated with amyloidosis - ? Degradative enzyme defects?
Classification of amyloidosis

1. According to the cause / associated disease.
2. Whether generalised or localised.
3. According to the chemical composition of the amyloid material deposited.
4. According to the sites of deposition of the amyloid.
Classification of amyloidosis

1. SYSTEMIC (generalised, involving several organ systems)
   a) Primary amyloidosis: associated with an immunocyte dyscrasia.
   b) Secondary amyloidosis: occurs as a complication of underlying chronic inflammatory or tissue destructive process.
   c) Hereditary / familial amyloidosis

2. LOCALISED (deposits are limited to a single organ, e.g. heart)
# Types of Amyloid

<table>
<thead>
<tr>
<th>Type of Amyloid</th>
<th>Associated Diseases</th>
<th>Amyloid Protein &amp; Precursor</th>
<th>Favoured Sites of Deposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generalised</td>
<td></td>
<td></td>
<td>(kidney), heart, tongue, g.i. tract, skin, nerve, joint</td>
</tr>
<tr>
<td>Immunocytic dyscrasias (primary amyloidosis)</td>
<td>multiple myeloma and other B-cell neoplasm with monoclonal gammopathy</td>
<td>AL (Amyloid Light chain) derived from immunoglobulin light chain mainly λ</td>
<td></td>
</tr>
<tr>
<td>Chronic inflammatory disease (secondary amyloidosis)</td>
<td>TB, leprosy, bronchiectasis, osteomyelitis rheumatoid arthritis, Crohn's and ulcerative colitis Ca kidney, Hodgkin's disease</td>
<td>AA (Amyloid Associated protein) derived from Serum Amyloid Associated protein (SAA)</td>
<td>kidney, liver, spleen, intestine, lymph nodes, adrenals</td>
</tr>
<tr>
<td>Hereditary syndromes</td>
<td></td>
<td></td>
<td>as for secondary amyloidosis</td>
</tr>
<tr>
<td>Familial Mediterranean Fever</td>
<td></td>
<td></td>
<td>peripheral and autonomic nerves</td>
</tr>
<tr>
<td>Neuropathic forms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemodialysis associated</td>
<td>chronic renal failure</td>
<td>β₂-microglobulin</td>
<td>synovium, joints and tendon sheaths; carpal tunnel syndrome</td>
</tr>
<tr>
<td>Localised</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Senile cardiac</td>
<td></td>
<td>Prelalbumin (transthyretin)</td>
<td>heart</td>
</tr>
<tr>
<td>Senile cerebral</td>
<td>Alzheimer's disease</td>
<td>A4 (β-protein) (no P component)</td>
<td>brain</td>
</tr>
<tr>
<td>Endocrine</td>
<td>e.g. medullary Ca thyroid</td>
<td>procalcitonin from calcitonin from insulin</td>
<td>in thyroid tumour</td>
</tr>
<tr>
<td></td>
<td>e.g. diabetes mellitus</td>
<td></td>
<td>islets of Langerhans</td>
</tr>
<tr>
<td>Isolated deposits</td>
<td>localised amyloid of lung, skin, genito-urinary tract etc.</td>
<td>AL from immunoglobulin light chains</td>
<td>lung, larynx, skin, g.u. tract, tongue etc.</td>
</tr>
</tbody>
</table>
Immunocytologic dyscrasias with amyloidosis (primary amyloidosis)

- AL amyloidosis complicates a proportion of all forms of monoclonal plasma cell proliferation, whether benign monoclonal gammopathy or myeloma (malignant).
- Usually systemic in distribution.
- Most common form of amyloidosis.
- Plasma cells produce abnormal amounts of a specific immunoglobulin (M-protein spike on serum electrophoresis), often with an excess of light chains (Bence-Jones protein) of either the kappa or lambda variety.
Immunocytoses with amyloidosis (primary amyloidosis)

- Found in mesenchymal tissues: heart (90% cases), kidneys, bowel, tongue, skin.
- Prognosis is poor; most commonly die of cardiac failure (restrictive cardiomyopathy; arrhythmias).
- Benign form:
  - No bony disease or plasmacytoma
  - Clinical effects derive from amyloid deposition without other associated disease.
  - Monoclonal Ig’s or free light chains (or both) can be found in the serum or urine (Bence-Jones proteinuria).
- Modest increase in the numbers of plasma cells in the bone marrow.
- Myeloma:
  - Destructive bony infiltrate of malignant plasma cells.
  - Multiple lytic skeletal lesions.
Reactive systemic amyloidosis (secondary amyloidosis)

- Amyloid deposits composed of AA protein.
- Associated with chronic inflammatory disease (infectious and non-infectious).
- Due to protracted breakdown of cells.
- At one time: TB, leprosy, bronchiectasis and chronic osteomyelitis were most NB underlying conditions.
- Now: rheumatoid arthritis (3%), ankylosing spondylitis and inflammatory bowel disease (Crohn disease and ulcerative colitis), dermatomyositis.
- Heroin abusers injecting subcutaneously “skin popping”.

Heroin abusers injecting subcutaneously “skin popping”.


At one time: TB, leprosy, bronchiectasis and chronic osteomyelitis were most NB underlying conditions.
Reactive systemic amyloidosis
(secondary amyloidosis)

- In Western world, RA is commonest cause.
- Distribution: many parenchymal organs: kidney, liver, spleen, intestines, adrenals.
- Patients present with proteinuria, nephrotic syndrome and hepatosplenomegaly.
- Renal failure is the most common cause of death.
Heredofamilial amyloidosis

- A variety of forms. Most rare.
- Occur in limited geographic areas, groups.
- A group of autosomal dominant familial disorders is characterised by deposition of amyloid in the nerves (peripheral and autonomic): familial amyloidotic polyneuropathies. Fibrils are made up of mutant transthyretins (ATTR).
- Familial Mediterranean fever:
  - autosomal recessive; febrile disorder of unknown cause.
  - Affects Sephardic Jews, Arabs, Armenians.
  - characterised by attacks of fever accompanied by inflammation of serosal surfaces (peritoneum, pleura, synovium).
  - Widespread tissue involvement, indistinguishable from systemic amyloidosis.
  - Fibrils made up of AA proteins.
Haemodialysis-associated amyloidosis

- Deposition of $\beta_2$-microglobulin occurs in patients on long-term haemodialysis for renal failure.
- $\beta_2$-microglobulin present in high concentrations in patients with renal disease; retained as it is not filtered by dialysis machines.
- Amyloid deposits in the synovium, joints and tendon sheaths.
- Carpal tunnel syndrome a common complication.
Localised amyloidosis

- Sometimes amyloid deposits limited to a single organ / tissue; these have a firm, waxy cut surface.
- Deposits may produce grossly detectable nodular masses, or be evident only on microscopic examination.
- **Kidneys**: deposits in mesangial area of glomerulus and small arteries; enlarged in early stage and progressive reduction in size.
- **Heart**: individual fibres cased; involvement of walls of small coronary arteries; endocardium – pin-point nodules in wall of left atrium.
- **Liver**: enlarged, pale, firm. Cut edge is sharp; amyloid in walls of sinusoids (space of Disse). Minimal functional effects.
- **GIT**: in muscle coats and walls of small vessels; bowel rigid; malabsorption and bleeding may occur.
- **Spleen**: deposits in walls of small vessels and muscle coats. May have a follicular distribution (sago spleen), or more diffusely in the walls of splenic sinuses (lardaceous spleen).
- **Lung / bronchial**: may present as large, obstructing masses.
Endocrine Amyloid

- Microscopic deposits of localised amyloid may be found in certain endocrine tumours.
- Medullary carcinoma of thyroid, islet cell tumours of pancreas, pituitary adenomas, phaeochromocytomas.
Lauren's pituitary adenoma.
Amyloid of aging

- Several well-documented forms associated with aging.
- Senile systemic amyloidosis:
  - systemic deposition in elderly pts
  - dominant involvement and dysfunction of heart.
  - Amyloid composed of normal TTR molecule.
Diagnosis:

- By histopathological means.
- Sites of biopsy if amyloid suspected:
  - Rectal
  - Renal (esp. if proteinuria)
  - Gingival
  - Fat aspiration
References:


3. “Robbins and Cotrane Pathological Basis of Disease”, 7th Edition; Kumar, Abbas, Fausto; Elsevier; 2005