Amyloidosis

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Goals:
To explain how amyloid forms and what diseases are caused by it

Objectives
1. Explain why amyloid forms
2. When to suspect amyloidosis
3. How to diagnose amyloidosis
4. What are the treatment options

Outline:

1. Amyloid: what is it and why it forms
2. Major types of amyloid
3. Clinical presentation
4. Pathogenesis and pathology
5. Diagnosis: generic and type specific
6. Treatments
7. Amyloid beyond disease

Robbins pages 182-187

Amyloid: what is it and why does it form?

The term "amyloid" is actually a misnomer. It means starch. Indeed originally, in the late 18th century, it was felt that these peculiar tissue deposits were composed of starch based on their reaction with iodine. However, subsequent studies clearly showed that deposits of amyloid contained predominantly protein.
Tissue deposition of amyloid causes diseases termed amyloidoses.

The essence of amyloid formation is protein mis-folding. During this process a protein undergoes a conformational change, during which its secondary structure acquires a beta-pleated sheet conformation.
Normal, physiologic proteins when properly folded are typically composed of a mixture of segments in different folds, including alpha-helix and beta-pleated sheets. The latter, being hydrophobic, are typically not exposed to the aqueous milieu, in order to preserve protein's solubility.

Consequent to such mis-folding, protein becomes hydrophobic, insoluble, non-functional and resistant to degradation; the presence of extensive beta-pleated sheets makes the protein "sticky", i.e. readily aggregating with other similar protein molecules. Deposits of amyloid are typically formed extracellularly. The presence of beta-pleated sheets makes the protein Congophilic with display of a green color under polarized light. The latter feature is diagnostic of amyloid.
Deposits of amyloid at high magnification, such as that obtainable with the electron microscope,

have a fibrillary structure, forming non-branching rigid fibrils with width ranging between 8-12 nm. Such fibrils are also diagnostic of amyloid.

Amyloidogenesis – the concept

Under normal circumstances, a small fraction of proteins produced by a cell may undergo mis-folding. This process is, however, under the control of protein quality control systems, which are either: intracellular (proteasomes) or extracellular (macrophages). Thus, such mis-folded proteins are quickly eliminated and either broken down and "recycled" back into the synthesis of new proteins or disposed of as various membrane-bound inclusion bodies. However, under certain situations, the protein quality control system may become ineffective in preventing mis-folding.
Situations which facilitate mis-folding may include: increased concentration of certain proteins, production of proteins that are inherently less stable, mutations rendering affected proteins unstable, or generation of proteolytic cleavage products that are partially or completely resistant to complete proteolysis. Thus, the process of mis-folding is a protein recycling issue.
Proteins with a beta-pleated sheet conformation tend to aggregate, form protofibrils and finally "mature"fibrils. While only mature fibrils are fibrillary and congophilic, it is postulated that the intermediates are more proteo-toxic than mature fibrils. The pathogenicity of amyloid is not simply the consequence of tissue displacement or replacement by deposits. In recent years, it has become apparent that amyloid protein oligomers or protofibrils exert a greater direct toxic effect than the mature fibrils. Importantly, this may explain why patients who respond to therapy demonstrate clinical improvement despite their seemingly-unreduced load of amyloid deposits.

This concept underscores the importance of early diagnosis and the need to develop strategies for prevention of amyloidogenesis. The mis-folded protein may be subjected to a partial degradation.

As expected, the above-described process can affect several proteins, and indeed, currently >32 protein types have been shown to be involved in amyloid tissue deposition. While some of those proteins circulate in the blood and, hence, generate systemic deposits, in the case of local production of the precursor protein, deposits of amyloid develop and deposit only locally. Certain proteins, such as immunoglobulin light chains, when produced by the bone marrow plasma cells, readily enter the circulation and may cause systemic light chain amyloidosis. However, when plasma cells form extramedullary tumors (plasmacytoma) or tumor-like proliferation, their products are typically local and potential deposits of amyloid will be local in distribution. Other proteins, such as those produced by the liver (for example transthyretin), enter the circulation and, hence, the deposits of amyloid are typically systemic. Other proteins may be exclusively local and, hence, amyloid deposits are always localized (Alzheimer's diseases limited to brain).

Even when amyloid fibril precursor proteins circulate in the blood, the distribution of tissue deposits in different organs is not even. Thus, certain organs, in particular the kidneys, heart, and peripheral nerves tend to be affected more than other tissues. It is postulated that certain local factors, not fully understood, may facilitate amyloid deposition is such "preferred" organs.

A clinically important distinction is between diseases that are treatable versus those for which no treatments are currently available; diagnosis of hereditary amyloidoses is also important for counselling.

The classification (and nomenclature) of amyloidoses is based on the amyloid protein type. Thus "A" denotes amyloid and is followed by an abbreviation derived from the protein type.

Among the most common amyloidoses are those derived from the immunoglobulin light chain (AL) and amyloidosis associated with chronic inflammation – AA, which is derived from its soluble precursor protein- SAA. Systemic AL is the most common amyloidosis in the developed world while AA is a systemic amyloidosis that is much more frequently diagnosed in underdeveloped countries. However, the most prevalent amyloidosis worldwide is derived from beta protein, Aβ, which is associated with Alzheimer's disease and, as such, represents localized amyloidosis.
In this review, I will discuss systemic amyloidoses, focusing on early and precise diagnosis of the amyloid protein and the selection of available treatments.

1. Clinical presentation:
Systemic amyloidoses typically involve multiple organ systems, in particular the kidneys, the heart and peripheral nerves. With regard to renal involvement, the most typical presentation is with proteinuria/nephrotic syndrome, which should prompt a clinical workup for the underlying cause. Moreover, the differential diagnosis of proteinuria is relatively short and, hence, not surprisingly, amyloidosis is most frequently diagnosed by nephrologists. In contrast involvement of the heart and/or peripheral nerves is more difficult to diagnose correctly, in particular in the early stages. It is important to pay attention to some external signs that are individually relatively rare but can be very helpful when present. These include the following: macroglossia, periorbital purpura, submandibular swelling, shoulder pad, nail lesions.

2. Epidemiology: amyloidoses are relatively rare but are frequently underdiagnosed. It is estimated that the number of cases of systemic amyloidosis diagnosed in the US is probably <5,000 per annum.
3. Etiology/pathogenesis:
The central mechanism involved is that of protein mis-folding leading to a β pleated sheet conformation. However, different mechanisms are involved in different amyloid types depending on the type of protein. Thus, AL is associated with clonal plasma cells, which produce light chains and which are prone to undergo fibrillogennesis. AA amyloidosis is associated with an underlying chronic inflammation. In these two amyloidoses, the precursor protein is produced in increased amounts. In contrast, in hereditary amyloidoses, for example ATTR derived from a transthyretin mutant, precursor protein is produced in normal amounts but is amyloidogenic owing to its inherent instability consequent to germline mutation.

Several mechanisms have been proposed to explain why these amyloid deposits form, and it is possible that more than one mechanism may be involved concurrently. Thus, a sustained increase in the concentration of the precursor protein, proteolytic remodeling, or intrinsic protein instability, or instability due to a mutation, are all known mechanisms. These are accompanied

by failure or overload of the protein quality control system responsible for *in vivo* clearance and recycling of abnormally folded proteins. The latter may also explain why amyloidoses primarily affect older patients whose chaperone systems have been weakened by prior insults.

AL amyloidosis is derived from immunoglobulin light chain produced by a plasma cell clone. While a subset of AL (<15%) may be associated with multiple myeloma, the majority of cases (85%) are associated with plasma cell dyscrasia where the tumor burden is frequently low and its detection may require sophisticated laboratory methods. Hence, the latter cases used to be referred to as "primary" AL. It is postulated that such clones produce light chains that are particularly detrimental owing to acquired mutations impacting their physicochemical properties, rendering them amyloidogenic. Most cases of AL are derived from lambda light chain even though, normally, kappa light chain is more prevalent.
Most importantly, these "small dangerous clones" producing AL are ultimately lethal and must be eradicated by methods similar to those applicable to a large tumor burden. These methods involve intense chemotherapy (myeloablative melphalan with autologous stem cell transplantation) and, more recently, therapies targeting proteasomes and aggresomes. Hence, the correct diagnosis of the amyloid protein type is critical. This aggressive treatment has been shown to lead to durable responses and, in fact, survival in excess of 10 years can be achieved. The biggest problems are: (i) delayed diagnosis, which makes successful treatment difficult to achieve owing to advanced damage to target organs and (ii) patients with a low performance status not being able to tolerate such aggressive therapy and leading to unacceptable mortality rates.

Reactive systemic amyloidosis – AA amyloidosis
AA amyloid deposits are derived from SAA protein (serum amyloid A protein). The sustained elevation of SAA is associated with long standing inflammation and, hence, typically, AA is secondary to an associated inflammatory condition. In developed countries, these conditions include rheumatoid arthritis and inflammatory bowel disease (Crohn's diseases); also heroin abusers, "skin-poppers" may succumb to AA. In underdeveloped countries, chronic infections are the leading cause of AA. Amyloid deposits are systemic, with preferential involvement of kidneys, liver and spleen.
Interestingly, a subset of patients may develop f**amilial AA.** The best known is familial Mediterranean fever [FMF], which is an autosomal recessive autoinflammatory disease caused by mutations in the *MEFV* gene, which encodes pyrin and is involved in the regulation of innate immunity. In FMF, there is excessive production of cytokine IL-1 in response to inflammatory stimuli associated with episodic attacks of fever accompanied by inflammation of serosal surfaces such as pleuritis, peritonitis ["polyserositis"] and arthritis. FMF usually occurs in people of Mediterranean basin origin: Sephardic Jews, Armenians, Arabs, Greeks, Turks and Italians
FMF can be successfully treated with colchicine (produced from crocuses).

Hereditary amyloidoses
Several proteins with mutations have been shown to be associated with amyloidosis. The best known among these proteins is transthyretin. Transthyretin (TTR) is produced predominantly by the liver, with small amounts being produced by the choroid plexus and the eye. TTR is

involved in transport of thyroxin and retinoic acid. Many mutations have been identified, most of which are associated with amyloidosis, and the phenotype shows some variability depending on the mutation. Typically, there is polyneuropathy (familial amyloidotic polyneuropathy – FAP) with or without cardiac involvement while some patients have predominantly cardiac involvement. Interestingly, although inheritance is autosomal dominant, owing to variable penetrance, not every person inheriting a particular mutation develops amyloidosis or its onset and intensity may be variable.
In general, the ATTR phenotype may show some variability, in particular with late onset of clinically apparent disease. It is estimated that one particular mutation (V122I) is present in approximately 4% of African Americans and is associated with late onset (>60 years of age) cardiomyopathy leading to heart failure.

Interestingly, TTR wild type is also relatively unstable and, in older individuals, can lead to predominantly cardiac amyloidosis. This particular amyloidosis typically affects elderly males and since it affects predominantly the heart, has been termed "cardiac Alzheimer's". Many patients with ATTR (associated with either a mutant or wild type gene) may initially present with carpal tunnel syndrome.

Thus, in terms of pathogenesis, amyloidosis can be associated with either increased production of the precursor protein (AL, AA) or normal levels of production of a mutant protein.

Other amyloidoses:
Other systemic amyloidoses:
ALect2: amyloidosis derived from leukocyte chemotactic factor 2 was relatively recently discovered. It is a slowly progressing process, affecting predominantly the kidneys, and appears to preferentially affect Americans of Mexican origin. Its pathogenesis is unclear at the present time and there is no specific treatment. It is, however, very important not to mistake it for AL amyloidosis because of the associated implications in treatment. ALect2 may also be quite prevalent in other parts of the world, for example in Egypt.

Hemodialysis-associated amyloidosis is associated with accumulation of β2-microglobulin which is typically not effectively removed by dialysis. Thus, patients on long term hemodialysis may develop amyloid-associated periarthritis, carpal tunnel syndrome or tenosynovitis. Kidney transplantation is the best treatment and prevention option, although newer generation hemodialysis membranes are associated with a lower incidence of this type of amyloidosis.

Localized deposits of amyloid can be encountered in the lung, larynx, skin, and urinary bladder. These deposits can be multifocal (within a given organ system) forming, at times, tumor-like masses. The treatment is typically surgical even for recurrent lesions. It is needless to stress that distinction from systemic amyloidoses is critical. Most localized amyloid deposits are derived from the immunoglobulin light chain, which is produced by a local plasma cell clone.
Interestingly, rare cases of iatrogenic localized amyloid deposits may be associated with insulin injection sites.
Several endocrine organs may harbor deposits of amyloid that are derived from the locally-produced hormone; certain tumors may also harbor deposits of amyloid.

4. Pathology:
All deposits of amyloid, regardless of their origin, look similar, and in paraffin sections, have a homogeneous appearance; their fibrillary nature can only be demonstrated with electron microscope. Importantly, early deposits of amyloid may be inconspicuous. In routine practice, the diagnosis of amyloid, of any type, requires a special stain, namely Congo red. Since this stain is not routinely performed in pathology, a high index of suspicion is required to detect (in particular) early deposits. Thus, it is postulated that the most frequently involved tissues, such as kidney, myocardium and peripheral nerve biopsy, should be routinely examined by Congo red stain. For diagnosis of amyloid, green birefringence under polarized light is required. Deposits of amyloid are typically irregularly distributed and, hence, a negative result does not rule out amyloid, in particular, in the case of a limited tissue sample.

Differential diagnosis of proteinuria/nephrotic syndrome in adults involves essentially only 4 entities: Focal and Segmental Glomerular Sclerosis/Minimal change disease spectrum, membranous nephropathy, diabetes and amyloidosis!!!

5. Laboratory tests: since diagnosis of amyloidosis requires tissue, all other laboratory tests are used to support the diagnosis of amyloid and its type but not to make it. While the biopsy of an affected organ gives the highest yield, at times a biopsy of a surrogate site may be used. To this end, AL and AA, and to a certain extent also other amyloid deposits, are present in the abdominal subcutaneous fat. Hence, fat biopsy may be used to diagnose systemic amyloidosis.

6. Prognosis of amyloidosis is amyloid type and stage dependent; delay in diagnosis of amyloidosis is a major factor associated with poor prognosis.

7. Treatment is amyloid protein type dependent and, hence, diagnosis of the amyloid type is of critical importance in providing the appropriate treatment as well as in avoiding harm to the patient caused by inappropriate treatment.

Amyloid-type diagnosis has typically involved immunohistochemistry but, in recent years, proteomic amyloid typing has emerged. There are advantages as well as disadvantages to each method, which needs to be kept in mind. In immunohistochemistry, one needs to have the pertinent antibody and, thus, typically testing with a panel of antibodies is applied. Proteomic methods have been instrumental in the discovery of new amyloid types and in testing rare amyloid types. However, a major challenge of this method is the identification of small deposits of amyloid.

The rationale for the application of proteomics methods to amyloid typing lies in the relative abundance of amyloid proteins in tissue where, frequently, it is the *dominant* protein. Proteomic techniques include the following steps: sample preparation, protein extraction and digestion into peptide fragments, followed by their subsequent separation, measurement by mass spectrometry and protein identification by informatics.

The principal goal of diagnosis is the distinction between treatable versus non-treatable diseases; moreover, amyloidoses with a genetic component also require genetic counselling. Although relatively few amyloid types are found in the majority of patients, clinicians must also consider rare amyloidoses before a specific therapy can be implemented.
Treatment of AL amyloidosis is centered on the pathogenic plasma cell clone. In prior decades, aggressive chemotherapy with stem cell transplantation was successful; in more recent years, newer therapies targeting plasma cells have been applied such as aggresome and proteasome inhibitors. Treatment of AA amyloidosis has also undergone significant improvement with addition of DMRADs and, more recently biologics, into treatment protocols. Since, in several of the hereditary amyloidoses, the liver is either the exclusive, or predominant, source of the abnormal protein, liver transplantation has been offered to affected patients as a form of "surgical gene therapy". Currently, pharmacologic therapies are being tested in clinical trials for ATTR, both hereditary and wild type. In other amyloidoses, most notably systemic amyloidosis derived from the leukocyte chemotactic factor-2, ALECT2, no therapy is currently available. However, it is important not to misdiagnose these amyloidoses as AL since the instigation of therapy for AL can have grave consequences for the patient.

The big picture - where does amyloid fit?

Amyloid is not only associated with disease but is also involved in aging and degenerative disorders. In the most general terms, amyloid deposition can be considered as a failure to recycle defective proteins in order to dispose of them.
Amyloidogenesis is also successfully utilized in nature, including by humans, under physiologic circumstances. For example, a spider web is an example of an amyloid fold.
Amylome is the universe of proteins that are capable of forming amyloid-like fibrils. The term "functional amyloid" is used in reference to amyloid in nature.
Amyloid has been used in nature for functional purposes such as the high-density packing of amino acids. The amyloid fold has unique mechanical or chemical properties and plays a role in a highly adapted applications. Other examples of amyloid include: bacterial biofilm, fungal spore production, melanosome production in the skin, and hormone packing in the secretory granules of the endocrine system. The Barnacle, a marine crustacean, uses the amyloid fold to produce a natural adhesive that is stable in different environments, including fully submerged in seawater. Amyloid fibrils are also applicable as biotechnological role models.

Amyloid in medicine: early diagnosis of amyloidosis and its successful treatment depends on close collaboration between the clinician and the pathologist. This presentation is aimed at increasing awareness of amyloidosis and providing education about the available treatments.

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