

PROPATH

THE FOCUS

Immunohistochemistry

Immunohistochemistry in Amyloidosis

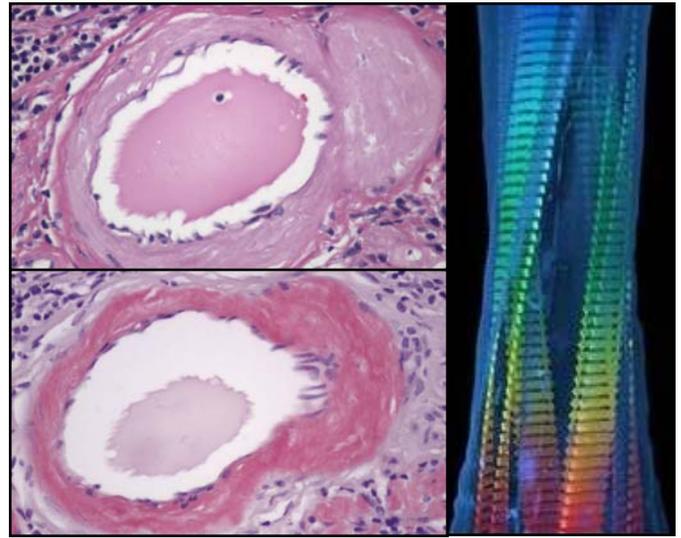
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Amyloid (a term meaning "starch-like") was named by Virchow in 1853, and refers to a family of abnormal proteins that share a common non-branching fibrillar "β-pleated sheet" structure, a configuration also found in natural silk. This β-pleated sheet configuration makes the proteins highly insoluble and resistant to enzymatic digestion, and is responsible for amyloid's staining characteristics ("waxy" amorphous homogeneous eosinophilic material on H&E and affinity for the azo dye Congo red) and optical properties ("apple green" birefringence of Congo red stains under polarized light). In addition to the fibrillar components of amyloid, all amyloid depositis contain non-fibrillar glycoprotein elements (responsible for the PAS positivity of amyloid), including glycosaminoglycans, apolipoprotein E (Apo E), and amyloid P component. Amyloid P component (also known as serum amyloid P or SAP) is a normal circulating α-1 glycoprotein that is resistant to protease digestion, and has a high affinity for the fibrillar component of amyloid. Because of the body's inability to degrade these proteins, they can accumulate and eventually cause organ dysfunction, in both localized and systemic fashions. A thorough review of amyloidosis is far beyond the scope of this newsletter, so I will limit this discussion to basic information, and discuss how several immunostains may assist in the classification of the most common types of amyloid that are encountered in routine pathology practice. Amyloid associated with tumors (e.g. amyloid in medullary thyroid carcinoma) is not considered here.

Although at least 25 different chemical types of amyloid have been described, only a few types account for the large majority of cases. The different types of amyloid can be grouped as follows:

1. AL (Primary) amyloidosis: This type is associated with plasma cell disorders (multiple myeloma, etc.), and the abnormal amyloid proteins are derived from immunoglobulin light chains (AL amyloid) or less com-



H&E (top left) and Congo-Red (bottom left) of amyloid deposits in a blood vessel. The right panel shows a fancy computer-generated rendering of the β-pleated sheet configuration of amyloid fibrils (courtesy of Google internet search).

monly immunoglobulin heavy chains (AH amyloid).

2. AA (Secondary) amyloidosis: This form is associated with some forms of cancer and a variety of chronic inflammatory conditions, including chronic infections (osteomyelitis, leprosy, bronchiectasis, decubiti, etc.), rheumatoid arthritis, and Crohn's disease. Familial Mediterranean fever (FMF) (an autosomal recessive disorder) is also associated with AA amyloidosis. In AA amyloidosis, the abnormal amyloid proteins are derived from serum amyloid A protein (SAA protein), a normal serum acute phase reactant.

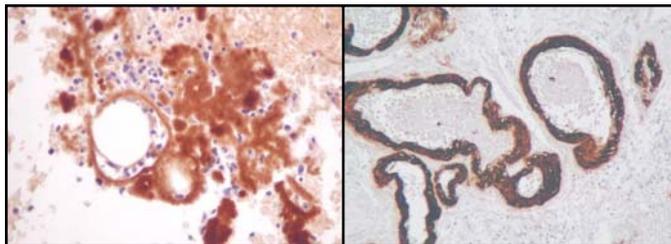
3. Amyloid β₂M - Dialysis-associated amyloidosis: This type of amyloid is found in some patients on chronic hemodialysis or peritoneal dialysis who develop amyloid deposits from accumulation of intact and modified β₂-microglobulins.

4. Hereditary amyloidoses: Hereditary amyloidoses are rare, but numerous familial types have been described that are typically transmitted in an autosomal dominant fashion. These diseases result from accumulation of amyloid derived from abnormal proteins produced secondary to hereditary mutations in genes coding for the proteins. The list of these abnormal "amyloidogenic" proteins include transthyretin (a.k.a. prealbumin) (ATTR amyloidosis), Apolipoprotein A1 (AApoA1 amyloidosis), Gelsolin (AGel amyloidosis), Lysozyme (ALys amyloidosis), Fibrinogen (AFib amyloidosis), Cystatin C (ACys amyloidosis), and others.

Precise chemical typing of amyloid proteins can be performed in formalin-fixed paraffin-embedded material, but those techniques are not widely available. Our approach to identification and typing of amyloid is fairly simplistic and easy to apply, and utilizes stains for **Congo Red** and **immunostains for amyloid P component and amyloid AA component**.

Although amyloid P component is present in all types of amyloid, it is important to know that it is also present in normal elastic tissue and basement membranes. **Therefore, a positive amyloid P immunostain does NOT necessarily mean that you are seeing "real" amyloid.** That is why it is **first necessary to establish the presence of amyloid by positive Congo red staining, BEFORE** proceeding to amyloid immunostains. Undoubtedly most pathologists are familiar with the descriptions of the classic "apple green" birefringence that is characteristic of amyloid when Congo red-stained sections are examined under polarized light. However, despite the fact that I am not colorblind, I have been singularly unimpressed with the ease of recognition of this finding, and if I look hard enough, I can see "apple green" birefringence in just about anything. I have had a much easier time identifying small or equivocal deposits of amyloid by examining Congo red-stained sections under a fluorescent microscope, where amyloid fluoresces a bright orange color. ProPath does a superb Congo red stain for those readers who may have a need for it.

Since amyloid P is present in all types of amyloid, it is always expected to be positive, and can help in highlighting the extent of deposition. AA component is present only in AA amyloidosis and FMF, so immunostains for AA component are positive only in those entities. β_2 M amyloid can be confirmed in dialysis patients by positive immunostains for β_2 -microglobulin. (Parenthetically, we do not have β_2 -microglobulin antibodies, because I have no positive control β_2 M amyloid tissue, although I would be grateful for any that a generous reader could provide.)



Immunostains for Amyloid P component (left) in a bone marrow biopsy from a patient with myeloma (AL amyloidosis), and a stain for amyloid AA protein in a kidney from a patient with rheumatoid arthritis (AA amyloidosis).

Being able to provide the clinician treating the patient with information on the type of amyloid can be of assistance in choosing the best options for therapy of patients with amyloidosis.

Immunostain results and their significance:

1. Amyloid P Positive, Amyloid AA Negative:

Possibilities include AL (Primary) Amyloidosis (plasma cell dyscrasias), β_2 M Amyloid (dialysis-associated), and Hereditary amyloidoses.

2. Amyloid P Positive and Amyloid AA Positive:

Characteristic of AA (Secondary) Amyloidosis (chronic infection, rheumatoid arthritis, Crohn's, Familial Mediterranean Fever, some cancers).

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