



Immunology 2017: handout 18

Amyloidosis

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INTRODUCTION

This handout covers an important topic that you need to understand because it will come again in many systems (CVS, GUS, CNS) and it has many applications in your clinical years. We decided to put it in your immunology course curriculum because many diseases that cause amyloidosis are immunologic in nature as you will see.

Amyloidosis is a condition, a group of disorders (not a single disease) that is associated with a number of inherited and inflammatory disorders, some of which are immunologic in nature, in which extracellular deposits of fibrillar proteins are responsible for tissue damage and functional compromise.

These abnormal fibrils are produced by the aggregation of misfolded proteins (which are soluble in their normal folded configuration).

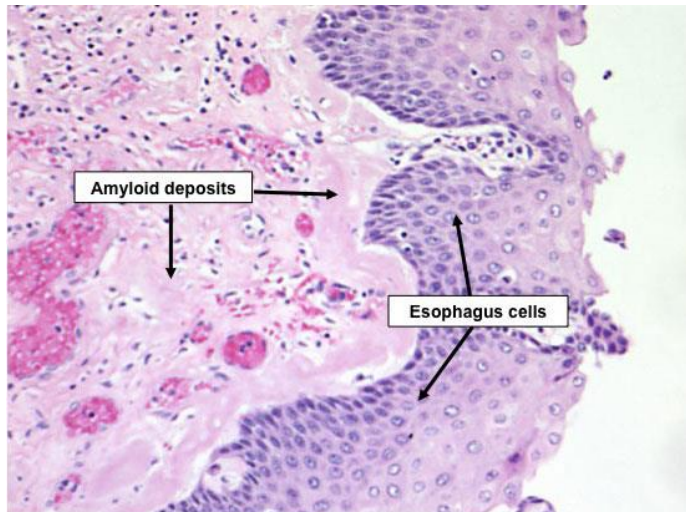
These fibrillar deposits bind glycoproteins like proteoglycans and glycosaminoglycans, including heparin sulfate and dermatan sulfate, and plasma proteins, mainly serum amyloid P component (SAP). These proteoglycans contain *sugar groups* that give the amyloid deposits staining characteristics which were initially thought to resemble starch (amylose). Therefore, the deposits were called amyloid, although the deposits are unrelated to starch.

DIAGNOSS

Amyloidosis is diagnosed on morphologic grounds.

With the light microscope and hematoxylin and eosin stains, amyloid appears as an amorphous, eosinophilic, hyaline, extracellular substance.

Special stains are used to demonstrate that this amorphous hyaline substance is amyloid, and no other fibers like collagen. The most widely used stain is the Congo red stain, which under ordinary light gives a pink or red color to tissue deposits, but with polarized microscopy this stain gives a specific green birefringence of the amyloid which differentiates it from collagen and other substances.



This is an H & E picture showing amyloid deposits as amorphous eosinophilic substance.

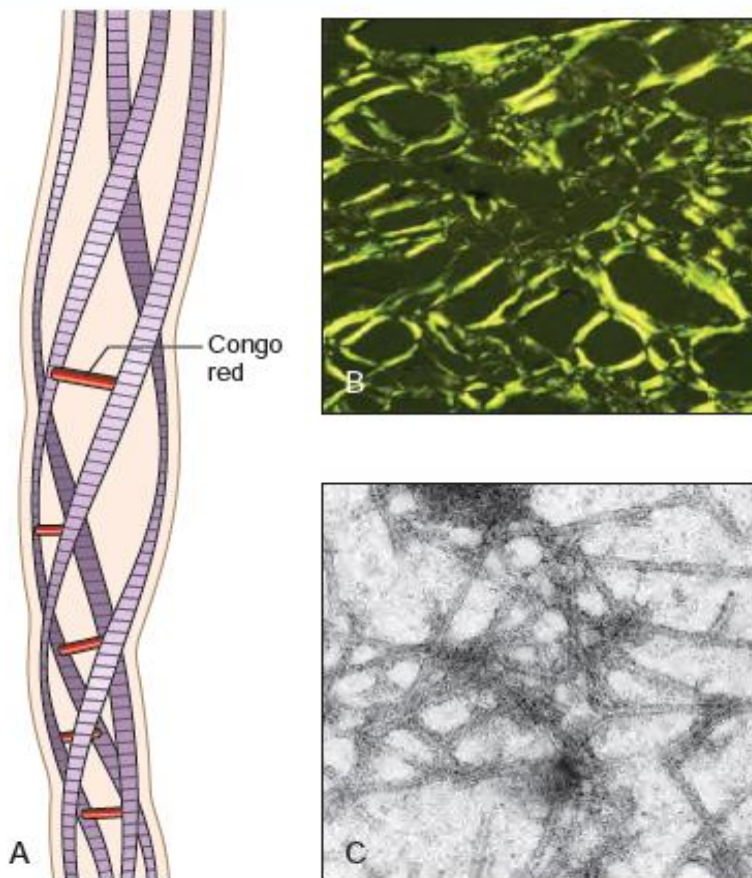


Figure 6-44 Structure of amyloid. A, A schematic diagram of an amyloid fiber showing four fibrils (there can be as many as six in each fiber) wound around one another with regularly spaced binding of the Congo red dye. **B,** Congo red staining shows apple-green birefringence under polarized light, a diagnostic feature of amyloid. **C,** Electron micrograph of 7.5- to 10-nm amyloid

PROPERTIES OF AMYLOID PROTEINS

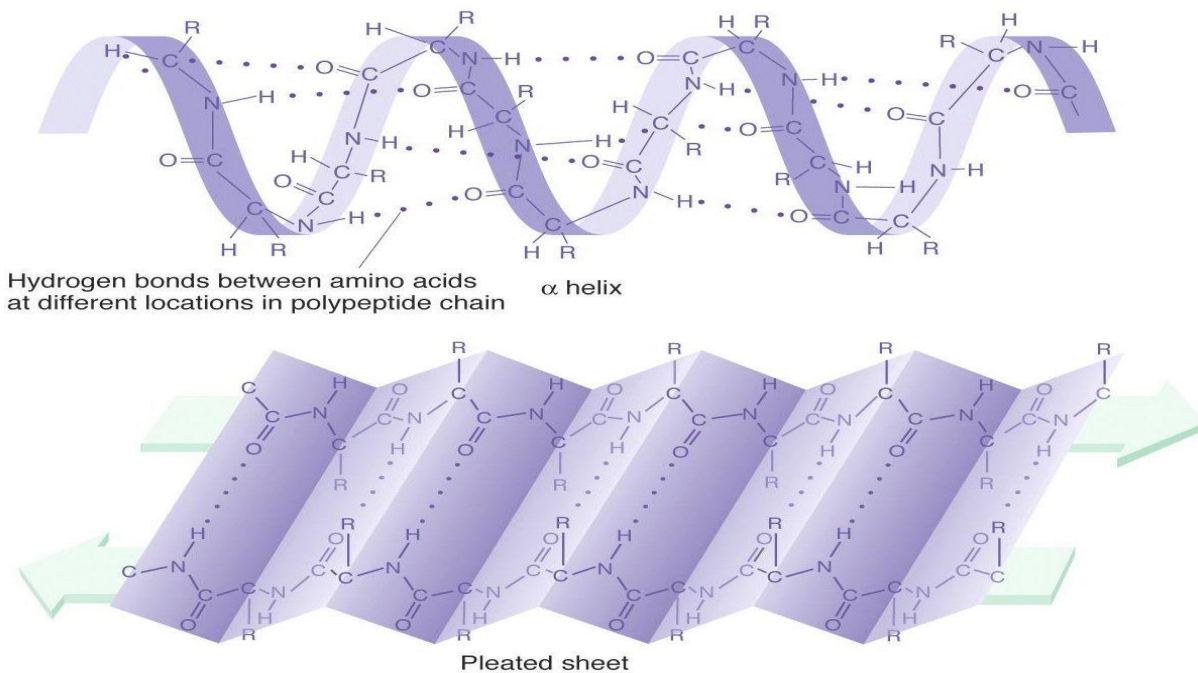
All amyloid deposits have a similar appearance and staining characteristics, but amyloid is **not** a single chemical entity. There are more than 20 different proteins can aggregate and form fibrils with the appearance of amyloid.

SO amyloid proteins are different chemically, but all have the same physical characteristics.

Physical Nature of Amyloid: By electron microscopy, all types of amyloid consist of *continuous, non-branching fibrils* with a *diameter* of approximately 7.5 to 10 nm. They all have characteristic *cross- β -pleated sheet* conformation. ***This conformation is seen regardless of the clinical setting or chemical composition and is responsible for the distinctive Congo red staining and birefringence of amyloid.***

Please remember what you already know from biochemistry : The β - sheet is a secondary structure in proteins that consists of beta strands (polypeptide chain around 3 to 10 amino acids) connected laterally by at least two or three hydrogen bonds, forming a twisted, pleated sheet.

You “hopefully” know that the other major secondary protein structure is the alpha helix!



Chemical Nature of Amyloid:

As we said amyloid proteins are a heterogeneous group of proteins that share the physical but not the chemical characteristics. There are three common forms of amyloid:

1. **The AL (amyloid light chain)** protein is made up of complete immunoglobulin light chains, the amino terminal fragments of light chains, or both. Most of the AL proteins are composed of **λ light chains** or their fragments, but **κ chains** are present in some cases.

The amyloid fibril protein of the AL type is produced from **free Ig light chains secreted by a monoclonal population of plasma cells, and its deposition is associated with certain forms of plasma cell tumors.**

2. **The AA (amyloid-associated):** this type of amyloid fibril protein is derived from a non-Ig protein made by the liver called SAA (serum amyloid-associated) protein that is synthesized in the liver and circulates bound to high density lipoproteins. The production of SAA protein is increased in **inflammatory** states as part of the **acute phase response**; (you should remember this, I taught you inflammation!!!!) therefore, this form of amyloidosis is **associated with chronic inflammation**, and is often called secondary amyloidosis.

3. **β -amyloid protein ($A\beta$)** constitutes the core of **cerebral plaques found in Alzheimer**. The $A\beta$ protein is derived by proteolysis from a much larger transmembrane glycoprotein, called amyloid precursor protein. (I'll ask you about this next semester in the CNS)

The above three are the main types of proteins in amyloidosis, but there are also other types like:

4. **Transthyretin (TTR)** is a normal serum protein that binds and transports thyroxine and retinol. Several mutant forms of TTR (and its fragments) are deposited in a group of genetically determined disorders referred to as **familial amyloid polyneuropathies**.

Normal TTR is also deposited in the heart of aged individuals (senile systemic amyloidosis).

5. **β 2-microglobulin**, a component of MHC class I molecules. it is found in amyloidosis that complicates **long-term hemodialysis**.

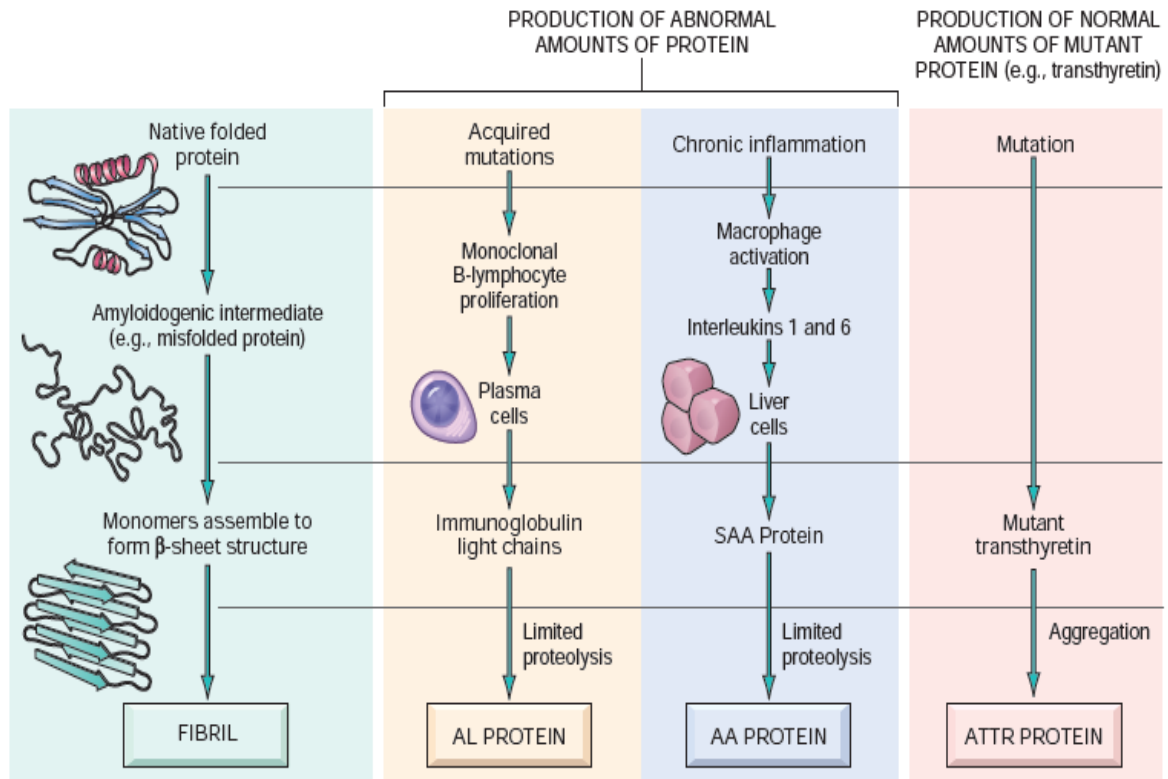


Figure 6-45 Pathogenesis of amyloidosis, showing the proposed mechanisms underlying deposition of the major forms of amyloid fibrils.

Pathogenesis and Classification of Amyloidosis

Amyloidosis results from **abnormal folding** of proteins, which become *insoluble, aggregate, and deposit* as fibrils in *extracellular* tissues.

Normally, misfolded proteins are degraded intracellularly in proteasomes, or extracellularly by macrophages. However, in amyloidosis, these mechanisms fail, leading to accumulation of a misfolded protein outside cells.

Amyloid may be **systemic** (generalized), involving several organ systems, or it may be **localized**, when deposits are limited to a single organ, such as the heart or kidney.

On clinical grounds, the systemic, or generalized, pattern is subclassified into **primary** amyloidosis, when it is associated with plasma cell disorder, or **secondary** amyloidosis, when it occurs as a complication of an underlying chronic inflammatory or tissue-destructive process.

Primary Amyloidosis: which is systemic ,AL type, and associated with plasma cell disorders (disorders, including neoplasms but not just neoplasms. More details below)

This is the most common form of amyloidosis. It occurs in 5% to 15% of individuals with multiple myeloma.

The malignant plasma cells synthesize abnormal amounts of a single Ig (monoclonal gammopathy), producing an M (myeloma) protein spike on serum electrophoresis. In addition to the synthesis of whole Ig molecules, the malignant plasma cells also secrete free, unpaired κ or λ light chains (referred to as Bence-Jones protein). These may be found in the serum, and due to their small molecular size, Bence-Jones proteins are excreted and concentrated in the urine.

In primary amyloidosis, the free light chains are not only present in serum and urine but are also deposited in tissues as amyloid.

Please note, however, that the great majority of myeloma patients who have free light chains in serum and urine do not develop amyloidosis.

So why some light chains deposit and others do not?? it is believed that the ability of these chains to accumulate and deposit depends on their specific amino acid sequence.

PLEASE NOTE THAT Most persons with AL amyloid **do not have classic multiple myeloma**, however, monoclonal immunoglobulins or free light chains, or both, can be found in their serum or urine. Most of these patients have a modest increase in the number of plasma cells in the bone marrow, which secrete the precursors of AL protein. Thus, these patients have an **underlying monoclonal proliferation of plasma cells (monoclonal gammopathy) in which production of an abnormal protein, rather than production of tumor masses, is the predominant manifestation.**

Secondary: Reactive Systemic Amyloidosis.

The amyloid deposits in this pattern are systemic and composed of AA protein. It is secondary to an **associated inflammation**. Previously, Tuberculosis, bronchiectasis, and chronic osteomyelitis were the most important underlying conditions, but with effective antibiotics these conditions decreased. More commonly now, reactive systemic amyloidosis complicates **rheumatoid arthritis**, ankylosing spondylitis, and inflammatory bowel disease. It can also occur in association with tumors like renal cell carcinoma and Hodgkin lymphoma.

In this form of amyloidosis, SAA synthesis by liver cells is stimulated by cytokines such as IL-6 and IL-1 that are produced during inflammation; thus, long-standing inflammation leads to a sustained elevation of SAA levels.

Please note that increased production of SAA by itself is **not** sufficient for the deposition of amyloid, because SAA is degraded to soluble products by the action of monocyte-derived enzymes. So: individuals who develop amyloidosis have an *enzyme defect* that results in incomplete breakdown of SAA or a genetically determined *structural abnormality* in the SAA molecule that make it resistant to degradation by macrophages.

Inherited familial Amyloidosis:

There is a variety of familial forms of amyloidosis, the most common is **familial Mediterranean fever** which is an autosomal recessive disease you will see in the pediatric rotations as it is common among Arabs.

This is an “**autoinflammatory**” syndrome associated with **excessive production of the cytokine IL-1** in response to inflammatory stimuli. Patients have **fever accompanied by inflammation of serosal surfaces**, including peritoneum, pleura, and synovial membrane (that’s why patients have abdominal pain and joint pain)

The gene for familial Mediterranean fever encodes a protein called pyrin (for its relation to fever), which is one of a complex of proteins that regulate inflammatory reactions via the production of proinflammatory cytokines . The amyloid fibril proteins deposited in this disease are made up of AA proteins (because the amyloid here is deposited due to inflammation, so you can think of it as secondary, reactive amyloidosis that is caused by a genetic defect)

Hemodialysis-Associated Amyloidosis.

Patients on longterm hemodialysis for renal failure can develop amyloidosis as a result of deposition of β_2 -microglobulin. This protein is present in high concentrations in the serum of persons with renal disease and in the past, it was retained in the circulation because it could not be filtered through dialysis membranes.

With new dialysis filters, the incidence of this complication has decreased substantially.

Localized Amyloidosis.

Sometimes, amyloid deposits are limited to a single organ or tissue without involvement of any other site in the body. The deposits may produce grossly detectable nodular masses or be evident only on microscopic examination. Nodular deposits of amyloid are most often encountered in the lung, larynx, skin, urinary bladder, tongue, and the region about the eye.

Frequently, there are infiltrates of lymphocytes and plasma cells in the periphery of these amyloid masses. At least in some cases, the amyloid consists of AL protein and may therefore represent a localized form of plasma cell-derived amyloid.

Endocrine Amyloid.

Microscopic deposits of localized amyloid may be found in certain endocrine tumors, such as medullary carcinoma of the thyroid gland , islet tumors of the pancreas, pheochromocytomas, and undifferentiated carcinomas of the stomach, and in the islets of Langerhans

Amyloid of Aging.

Several forms of amyloid deposition occur with aging. Senile systemic amyloidosis refers to the systemic deposition of amyloid in elderly patients (usually in their 70s and 80s). Because of the dominant involvement and related dysfunction of the heart, this form was previously called senile cardiac amyloidosis. Patients present with a restrictive cardiomyopathy and arrhythmias The amyloid in this form is derived from normal TTR.

Amyloid Protein	Protein Precursor	Systemic/ Localized	Related Disease
AA	Serum Amyloid A	S	Chronic inflammation or infection
AL	λ or κ light chains	S, L	Plasma cell clone related
ATTR	Transthyretin (TTR)	S	Familial, related to TTR mutations Old age, related to wild-type TTR
A β	A β protein precursor	L	Alzheimer's disease Cerebral amyloid angiopathy
A β_2 M	Beta $_2$ -microglobulin	S	Chronic hemodialysis

MORPHOLOGY OF AMYLOIDOSIS

In amyloidosis secondary to chronic inflammatory disorders, kidneys, liver, spleen, lymph nodes, adrenals, and thyroid are mainly affected.

Although amyloidosis associated with plasma cell proliferations cannot reliably be distinguished from the secondary form by its organ distribution, it more often involves the heart, gastrointestinal tract, respiratory tract, peripheral nerves, skin, and tongue.

In familial Mediterranean fever the amyloidosis may be widespread, involving the kidneys, blood vessels, spleen, respiratory tract, and (rarely) liver.

I don't want you to memorize these, just make sure you understand that the distribution is systemic, is variable, can affect any organ and it cannot reliably predict the type.

When amyloid accumulates in large amounts, the organ affected is frequently **enlarged** and the tissue appears **gray with a waxy, firm consistency**.



Liver affected by amyloidosis

Histologically, the amyloid deposition is always extracellular and begins between cells, often closely adjacent to basement membranes. As the amyloid accumulates, it encroaches on the cells, in time surrounding and destroying them.

The histologic diagnosis of amyloid is based almost entirely on its staining characteristics. The most common staining technique uses the dye Congo red, which under ordinary light imparts a pink or red color to amyloid deposits. Under polarized light the Congo red-stained amyloid shows so-called apple-green birefringence). *This reaction is shared by all forms of amyloid and is caused by the crossed β -pleated configuration of amyloid fibrils.* Confirmation can be obtained by electron microscopy, which reveals amorphous nonoriented thin fibrils. AA, AL, and ATTR types of amyloid can also be distinguished by specific immunohistochemical staining.

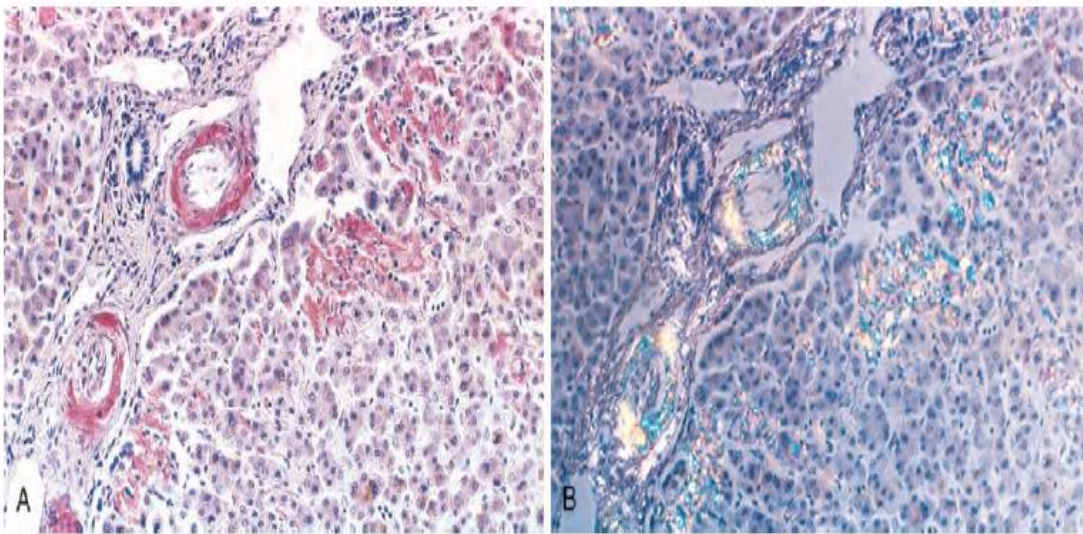


Figure 6-46 Amyloidosis. **A**, A section of the liver stained with Congo red reveals pink-red deposits of amyloid in the walls of blood vessels and along sinusoids. **B**, Note the yellow-green birefringence of the deposits when observed by polarizing microscope. (Courtesy Dr. Trace Worrell and Sandy Hinton, Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas.)

CLINICAL FEATURES.

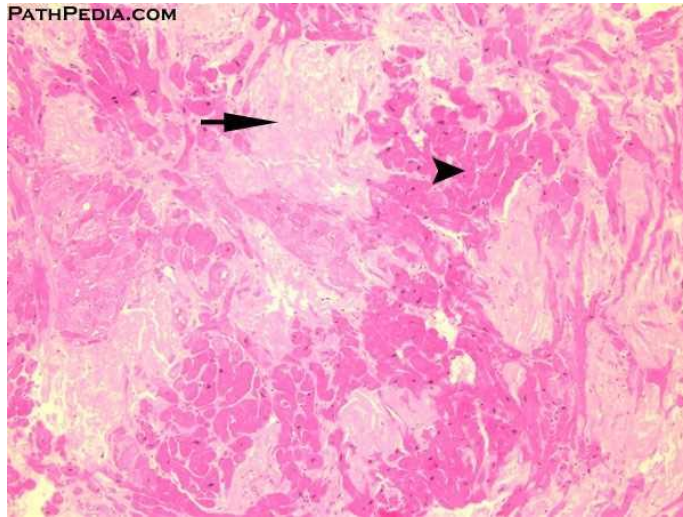
Amyloidosis may be found as an unsuspected anatomic change, with no clinical manifestations, or it may cause serious clinical problems and even death. **The symptoms depend on the magnitude of the deposits and on the sites or organs affected.**

Clinical manifestations at first are often nonspecific, such as weakness, weight loss, light-headedness, or syncope. Specific findings appear later and most often relate to renal, cardiac, and gastrointestinal involvement.

Renal involvement gives rise to **proteinuria** that may be severe enough to cause the **nephrotic syndrome**. This leads to renal failure and uremia. Renal failure is a common cause of death.

Cardiac amyloidosis may present as an insidious **congestive heart failure**. The most serious aspects of cardiac amyloidosis are conduction disturbances and **arrhythmias**. Occasionally, cardiac amyloidosis produces a **restrictive** pattern of cardiomyopathy.

Pic: cardiac amyloidosis



Gastrointestinal amyloidosis may be asymptomatic, or it may present in a variety of ways. Amyloidosis of the tongue may cause sufficient enlargement and inelasticity to hamper speech and swallowing. Depositions in the stomach and intestine may lead to malabsorption, diarrhea, and disturbances in digestion.

Vascular amyloidosis causes vascular fragility that may lead to bleeding, sometimes massive, that can occur spontaneously or following seemingly trivial trauma.

The prognosis for individuals with generalized amyloidosis is poor. Those with AL amyloidosis (not including multiple myeloma) have a median survival of 2 years after diagnosis.

Persons with myeloma-associated amyloidosis have an even poorer prognosis. The outlook for individuals with reactive systemic amyloidosis is somewhat better and depends to some extent on the control of the underlying condition.

Resorption of amyloid after treatment of the associated condition has been reported, but this is a rare occurrence. New therapeutic strategies aimed at correcting protein misfolding and inhibiting fibrillogenesis are being developed.

THANK YOU

