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# Amyloidosis - An Overview

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## Abstract

Amyloidosis is a heterogeneous disorder resulting from the deposition of toxic insoluble beta-sheet fibrillar protein aggregates. Amyloidosis can be acquired or hereditary. The disease can be localized or systemic. Amyloid can accumulate in the liver, spleen, kidney, heart, nerves, and blood vessels, causing different clinical syndromes. The variation in presentation and rarity of amyloidosis makes it a challenging diagnosis to and one third of cases are not diagnosed up till until a year after presentation. Ultrastructural examination and chemical analysis reveal the complex nature of amyloid. It emerges that on the basis of morphology and physical characteristics, all forms of amyloid are similar in appearance, but they are chemically heterogeneous. Based on analysis, amyloid is composed of 2 main types of complex proteins: Fibril proteins comprise about 95% of amyloid and non-fibrillar components which include P-component predominantly and there are several other different proteins which together constitute the remaining 5% of amyloid. Although not common, amyloidosis is a serious disease that results in significant mortality and morbidity. The most challenging issue is significant delay in diagnosis, sometimes up to several years. Early suspicion and thorough investigation are critical to making a timely diagnosis and referral for treatment.

**Keywords:** Amyloidosis; Plasma; Kidney; Heart

## Classification and terminology

It was Rudolph Virchow in 1854 who adopted the term "amyloid,". Amyloid deposits were noted by Rokitansky to have a "waxy" or "lardaceous" appearance grossly and by Virchow to be amorphous and hyaline on light microscopy. Amyloidosis is a result of extracellular tissue deposition of fibrils composed of low molecular weight in the range of 5 to 25 kD, many of these proteins circulate as constituents of plasma. Genetic factors, point mutations, deletions, and premature stop codons may result in structural changes predisposing to fibril formation (fibrillogenesis) by these proteins and therefore development of amyloid. Depending upon the type of amyloidosis, the actors that affect protein folding which includes chaperones protein and failure of disaggregating pathways, may be functional. These mechanisms also depend upon non-fibrillar components found in all types of amyloid, including serum amyloid P component (SAP), apolipoprotein E, glycosaminoglycans, and proteoglycans. At least 30 different human and 10 different animal protein precursors of amyloid fibrils are now known. The different clinicopathological types of amyloidosis is shown in table 1.

Amyloidosis may be localised amyloidosis where there is involvement of any one organ or systemic where there is involvement of two or more organs by amyloid protein deposits. Light chain amyloidosis (AL), or primary amyloidosis is the most prevalent type of amyloidosis in the developed countries which is due to plasma cell dyscrasia, is due to deposition of protein derived from immunoglobulin light chain fragments. In developing countries AA amyloidosis (secondary amyloidosis) related to chronic infection, is encountered more often. The nomenclature is based on amyloidogenic protein For eg:-AL (light chain), unmutated, Transthyretin (ATTR) in heart (age related senile amyloidosis) mutated forms of TTR, alpha chain of fibrinogen (Afib), Apolipoprotein AI, A II, (hereditary amyloidosis), AA amyloidosis (acute phase reactant

| Cliniopathological category | Associated disease | Major fibril Protein | Chemicaly related precursor protein |
|---|---|---|---|
| 1) Systemic amyloidosis | | | |
| Primary amyloidosis | Multiple myeloma and other monoclonal plasma cell proliferations | AL | Immunoglobulin light chains, chiefly λ type |
| Secondary amyloidosis | Chronic inflammatory conditions | AA | SAA |
| Hemodialysis-associated amyloidosis | Chronic renal failure | Abeta2 M | β2-microglobulin |
| 2) Herediatary amyloidosis | | | |
| Familial meditterian fever | | AA | SAA |
| Familial amyloidotic neutropathies | | ATTR | Transthyretin |
| Systemic senile neuropathies | | ATTR | Transthyretin |
| 3) Localised amyloidosis | | | |
| Senile cerebral | Alzheimer disease | Aβ | APP (amyloid precursor) |
| Endocrine | Type 2 diabetes | | AIAPP (islet amyloid pp) |
| Medullary carcinoma of thyroid | | ACal | Calcitonin |

**Table 1:** Clinicopathological types of Amyloidosis.

SAA) ALECT2 (Leucocyte cell derived chemotaxin 2). AL amyloidosis affects men slightly more often than women.The average age of diagnosed patients is 65 years and around 10% of patients are less than 50 years old.

## What defines an amyloid fibril protein-

An amyloid fibril protein is defined as follows:

- The protein must occur in body tissue deposits as rigid non branching fibrils of 10nm diameter
- Has affinity for Congo red and apple green birefringence when viewed with polarized light.
- The chemical identity of the protein must have been unambiguously characterized by protein sequence analysis
- Gene mutation as well as the variant amino acid sequence must be known whenever possible.
- When isolated from tissues and analyzed by X-ray diffraction, the fibrils exhibit characteristic cross b diffraction pattern.

## Clinical features in amyloidosis

Clinical manifestations depends on the number and extent of organ involvement but are usually not specific. Most common symptoms in systemic amyloidosis are weight loss, fatigue, edema and dyspnoea on exertion. The frequency of involvement is most commonly the heart 71%,followed by 58%, gastrointestinal tract (GI) 22%, nervous system 23%. Cardiac involvement is most common and it clinically it presents as fatigue, dyspnoea on exertion, peripheral edema, jugular vein distention, arrythmia and rarely MI. The Gold standard is endomyocardial biopsy and aTc-99m-pyrophosphate scintigraphy- ATTR patients. GI involvement presents as commonly as malabsorption, pseudo-obstruction, early satiety and weight loss. There maybe be hypoalbumnemia and anemia. 20% GI involvement are localized. Liver and spleen Involvement presents as hepatomegaly, elevated alkaline phosphatse and rarely hepatic rupture may occur. In splenic Involvement Howell Jolly bodies may be seen on peripheral smear. On gross spleen is firm and waxy consistency and patterns of involvement are two, where deposition may be in germinal centres this known as sago spleen and in sinusoids as which is known as lardaceous spleen. Nerve involvement presents as autonomic involvement bowel/bladder dysfunction, orthostatic hypotension, erectile dysfunction peripheral nervous system numbness, paraesthesia and pain. There is stocking-glove distribution with loss of vibration, absence of deep tendon reflexes. Pulmonary involvement commonly presents as submucosal deposition in tracheobronchial airway leading airway obstruction, hoarseness, segmental collapse, recurrent pneumonia. Diffuse interstitial disease, unilateral pleural effusion and nodular amyloidomas which are non-cavitary pulmonary nodules may be found but are however rare. Skin and soft tissue involvement pres-

ent as macroglossia, submandibular enlargement, spontaneous peri-orbital ecchymosis (raccoon eyes), nail dystrophy, hair loss, shoulder pad sign which is fluid in glenohumeral joint and synovial membrane deposition. There may be jaw claudication due to vascular involvement and carpal tunnel syndrome. The haematological manifestations include increased bleeding that may occur due to one or more of several causes, including reduced activity of factor X, vascular infiltration with amyloid, and abnormal liver function due to amyloid deposition. Other hematologic manifestations are related to the degree of organ involvement. These include anemia in patients with renal failure or multiple myeloma and thrombocytopenia due to splenomegaly.

In patients who have renal, cardiac, hepatic, and peripheral nerve amyloid, biopsy of the affected organs has a very high sensitivity of demonstrating amyloid. The first diagnostic studies that should be performed in patients with a compatible clinical syndrome and an immunoglobulin light chain abnormality are a subcutaneous fat aspirate and a bone marrow biopsy. Subcutaneous fat aspirate in an experienced laboratory will demonstrate amyloid deposits in 75% of patients tested. A protocol has been utilized in clinical studies which involves repeated aspirations carried out at sites about 10 cm lateral to the umbilicus using 10 mL syringes with negative pressure through a 16-guage needle.

The criteria for systemic amyloidosis is depicted in table 2.

When should we clinically suspect amyloidosis ?

| System involved | Criteria Biopsy of affected or alternate site AND | Tests required to demonstrate systemic involvement |
|---|---|---|
| Renal | Proteinuria of > 0.5 g/24 h (mainly albumin) | 24 h urine protein |
| Cardiac | Lab/clinical evidence of involvement<br>Echocardiograhy with more than 12 mm wall thickness in absence of other causes | Troponin I<br>N terminal pro brain natriuretic peptide<br>ECG showing low voltage (< 5 mm) in all 12 leads |
| Liver | Liver span > 15 cm in absence of heart failure<br>Alkaline phosphatase > 1.5 times | CT scan or radionuclide imaging<br>Alkaline phosphatase |
| Hepatic | Radiographic evidence of diffuse interstiial lung disease | CT scan/X ray |
| GI | amyloid deposits on biopsy | |

**Table 2:** Criteria for systemic involvement in amyloidosis.

- **Cardiac:** Infiltrative Cardiomyopathy –clinical spectrum from Fatigue to overt Congestive heart failure
- **Renal:** Albuminuria with or without renal insufficiency
- **Neuropathic:** Peripheral Neuropathy with demyelinating or axonal features/ autonomic neuropathy – othostatic hypotension, gastroparesis etc.
- **Hepatic:** Unexplained Hepatomegaly
- **Musculoskeletal:** Carpal Tunnel Syndrome
- **Skin and soft tissue:** Enlargement of the tongue
- **Gastro-Intestinal:** Weight loss associated, intestinal pseudo-obstruction, malabsorption
- Atypical myeloma

The diagnosis of amyloidosis once it is suspected is a four step approach. These steps are as follows :

- **Step 1:** Establish the presence of an amyloid-related systemic syndrome
- **Step 2:** Positive amyloid staining by Congo red in any tissue
- **Step 3:** Evidence of a monoclonal plasma cell proliferative disorder
- **Step 4:** Typing of amyloid using mass spectrometry – based proteomic analysis or Immune electron microscopy.

Step 1: To establish the presence of amyloid related syndrome baseline investigations like – hemogram, renal Function tests (RFT) Liver function tests(LFT) coagulation studies, electrocardiography (ECG), Serum protein electrophoresis (SPEP), Serum immunoelectrophoresis (sIFE), serum free light chain assay (sFLC) must be done. Assessment for Renal involvement would also include 24hr

urinary protein, urine SPEP and SIFE, renal biopsy. For assessing cardiac status trop T, NT-pro BNP, ECHO, Cardiac MRI, pyrophosphate scan, endomyocardial biopsy can be done as and where available. Hepatic involvement includes liver function tests and liver biopsy. To asses neuropathy nerve conduction studies (NCS) Electromyography (EMG), autonomic function tests can be carried out. Serum amyloid Protein (SAP) scintigraphy uses radiolabelled amyloid P component with I 123-SAP and a useful imaging agent for identifying amyloid deposits. The diagnostic sensitivity of serum amyloid P scintigraphy is 90% whereas diagnostic specificity is 93%. It however does not distinguish AL from other form of amyloidosis. The scintigraphy scans have shown that the distribution of amyloid within organs is non homogeneous and does not correlate well with clinical degree of organ dysfunction.

## Step 2

On hematoxylin and eosin staining (HE) amyloidosis appears amorphous eosinophilic acellular deposits. It can therefore be confused with collagen, fibrin, plasma, light chain deposits, heavy chain deposits therefore special stains are needed of which most important is Congo red. Congo Red was discovered by Herman Bennhold and the staining technique is named after him. The Selective Congo red binding to amyloid is attributed to non-ionic hydrogen bond between dye and amyloid and this stains amyloid as deep pink to red colour. The staining must be done on thick sections that are 6-10 microns thick. The alkaline pH enhances staining along with the high salt content. They are believed to depress dye ionization and electrostatic binding to non-amyloid structures. On polarizing microscopy apple green birefringence seen due to alignment of dye molecules on linearly arranged amyloid fibrils. It is important that yellow green birefringence of collagen be kept in mind. False positive staining by Congo red may be seen in elastotic dermis, deposits of lipid proteinosis, hyaline material of laryngeal, aural and nasal polyps and cauterized connective tissue. A salt concentration >50%in the stain or usage of Carnoys/Zenker-acetic, absolute alcohol as fixatives are more commonly related to nonspecific staining. Other stains that may be used are Thioflavin S or T which is more sensitive, but less specific and require a fluorescent microscope. Other stains are sulfated Alcian blue which is not specific stain, stain glycosaminoglycans. Crystal violet may be used but is less sensitive. Bone marrows for amyloidosis must be done if there is a clinical suspicion of amyloidosis in every case of Monoclonal gammopathy of undetermined significance(MGUS) or Multiple myeloma(MM) or Waldenstorm's macroglobulinemia (WM) and if there are increased clonal plasma cells in the bone marrow.

Diagnosis of MM associated AL amyloidosis is made when the criteria for both the conditions- namely AL amyloidosis and MM are met. Almost 18% patients with AL amyloidosis patients may show > 20% clonal plasma cells in bone marrow and in only 10–15% of the patients with MM concurrent diagnosis of AL amyloidosis is made at either during initial workup or sometime during the course of the myeloma. About 1/3rd of MM patients are found to have sub clinical amyloid deposits. This however has no impact on the toxicity and outcome of MM patients if occult, however if symptomatic amyloidosis clearly worsens their prognosis and is an alert for modification in therapy. In MM patients with atypical features i.e. with nephrotic range proteinuria, infiltrative cardiomyopathy, autonomic neuropathy, hepatomegaly and symptoms of partial bowel obstruction workup for amyloidosis must be taken up.

The differences between amyloidosis and MM are shown in table 3.

| Criteria | Amyloidosis | Multiple Myeloma |
|---|---|---|
| Plasma cell clone | 'small 'clone <10% | Is generally a large clone, median PC no >30% |
| Incidence rate | 8/million/year | Five times more common |
| Bone marrow | Minimally populated by plasma cells | Infiltrated and suppressed normal bone marrow |
| Organ involvement | Widespread organ involvement | Organ involvement rare |
| CRAB features | Absent | Present |
| Post transpant complications<br>Transplant related mortality | Organ failure common<br>11-25% | Infrequent<br>2% |

**Table 3:** Differences between amyloidosis and Multiple Myeloma.

## Step 3

Monoclonal Protein in AL Amyloidosis identification.

Serum M-component levels are low in amyloidosis and a paraprotein is detectable in the serum or urine by routine electrophoresis in approximately 50% of patients with AL amyloidosis. It is

essential to perform immunofixation because the level of paraprotein in AL amyloidosis is usually very low and routine electrophoresis is often negative. However even on immunofixation, no paraprotein is detectable in serum or urine in ~20% of cases.

Urine immunofixation is recommended when screening for AL amyloidosis because rare clonal dyscrasias may be missed using serum tests alone an M component can be detected in 99% of patients with AL amyloidosis by using the combination of sFLC analysis, serum protein electrophoresis (SPEP), and immunofixation electrophoresis (IFE). The difference between the amyloidogenic and uninvolved FLC concentration (dFLC) has become recognized as being useful in estimating the 'monoclonal' component and is applicable to patients with renal failure. 10–15% of AL patients have only minimally abnormal FLC and for these patients sFLC cannot be used for accurate monitoring. The most common serum immunofixation finding is free lambda chain. The median κ : λ ratio is 1 : 3.6. Kappa AL amyloid is associated with more GI and hepatic involvement, whereas lambda AL is associated more with renal involvement. Light chain deposition disease has a similar pathogenesis and shares some clinical manifestations with AL amyloidosis; the primary difference is that deposited light chain fragments generally do not form fibrils and do not have deposition of amyloid cofactors.

### Step 4

Typing of amyloid.

In IHC primary antibodies against various components of amyloid are used, while Ab against amyloid P component merely detects amyloid. Antibodies against AA stains only secondary amyloid cases.Ab against TTR, apo A I, fibrinogen (hereditary amyloidosis) stain strong and even while spotty staining may be seen in other amyloids. Strong and even immunostaining of the entire amyloid deposit by one non-anti-AL Ab is categorized as non-AL fibril. IHC has poor specificity (<50%) in light chain amyloidosis. Antigen masking, light chain fragmentation during amyloid fibril formation and variation in tissue processing- sub-optimal staining may complicate IHC typing. Also two or more amyloid fibril protein existence in same patient may complicate IHC typing further. Immunoelectron Microscopy(IEM) is a combination of EM and IHC, primary Ab binding to amyloid, followed by secondary Ab bound to colloidal gold. Gold-labelled secondary Ab localize s to amyloid and reduce background staining. IEM has a specificity of more than 99%.

On electron microscopy haphazardly distributed, non-branching solid fibrils with mean diameter of 10n are seen. Electron microscopy is however not specific as it can be seen in fibrillary deposition in fibrillary GN, glomerular sclerosis, fibronectin glomerulopathy and therefore comparison with light microscopy and Congo red is necessary. The typing of amyloid may be done by four techniques (a) Immunofluorescence (IF) (b) Immunohistochemistry (IHC), (c) Immunoelectron microscopy and Proteomics. IF is widely used in renal biopsy as frozen sections are available. In this fluorochrome labelled monoclonal antibodies against immunoglobulin components, seen under fluorescent microscope. Paraffin embedded tissues cannot be used. Mass spectroscopy using LD-MS/MS (laser dissection-tandem mass spectrometry) and MALDI-TOF are the two mass technologies that can be used to type the amyloid deposits. The limitations are that a given protein can only be detected if peptide fragment of appropriate size generated after enzyme digestion and it may be difficult to detect low abundance proteins/peptides as signals from these peptides may be buried among massive amount of information obtained from more abundant protein and MS simply may not able to scan them. The advantages are that it helps in global identification of protein and also helps in discovery of unsuspected proteins.

Diagnostic Criteria for AL amyloidosis – Mayo clinic/International Myeloma working group (IMWG) (All four criteria must be present)

- Presence of amyloid-related syndrome
- Positive Congo red staining or presence of amyloid fibrils on EM
- Evidence that amyloid is light chain related established by direct examination on spectroscopy-based proteomics or IEM
- Evidence of monoclonal plasma cell proliferative disorder (serum M protein, abnormal sFLC or clonal PC on BM)

### Cytogenetics in BM/amyloid tissue

CTG is an important prognostic factor, may help in therapeutic decisions. The most frequent genetic abnormalities in AL amyloidosis are t(11; 14) (50%), monosomy 13/del(13q) (36%), and trisomies (26%). t(11;14) has a poor treatment response to Bortezomib. Trisomies have shorter overall survival t(4; 14) and t(14; 16) were rarely found in AL, accounting only for 3 and 4% of patients respectively. Del 17p is found in 03% patients. Bortezomib abrogate the poor prognosis associated with above high risk CTG. Gain

of 1q21(<20%) has no adverse prognosis if treated with bortezomib.

### Prognosis and treatment

The prognosis of immunoglobulin light-chain amyloidosis has been associated with abnormalities in lactate dehydrogenase, β2-microglobulin, genetics, circulating plasma cells, the fraction of plasma cells in S-phase, and the serum amyloid P scanning technique. The simplest and most powerful assessments of prognosis revolve around the importance of cardiac amyloid in determining outcome. Patients are assigned a score of 1 point for each of the following:

- Difference between involved and uninvolved free light chains (FLC-diff) ≥18 mg/dL
- Cardiac troponin T (cTnT) ≥0.025 ng/mL
- N-terminal pro-B-type natriuretic peptide (NT-proBNP) ≥1800 pg/mL.

This creates stages I–IV with scores of 0–3 points. The median survival in months for these stages is 5tage I. 94.1 mnths, Stage II. 40.3 mnths, Stage III. 14 mnths, Stage IV. 5.8 mnths.

Treatment of the different types of amyloidosis generally varies with the cause of fibril precursor production. As examples, therapy is aimed at the underlying infectious or inflammatory disorder in secondary (AA) amyloidosis, at the underlying plasma cell dyscrasia in primary (AL) amyloidosis, and at either altering the mode of dialysis or considering a renal transplant in patients with dialysis-related amyloidosis. Liver transplantation may be effective in certain of the hereditary amyloidoses. Strategies to facilitate the clearance of amyloid deposits in tissue are also in development. A number of novel approaches to treatment are being investigated-they include agents that interfere with fibril formation; that inhibit the production of amyloidogenic precursors e.g., transthyretin [TTR], SAA, that neutralize oligomers or nonamyloid aggregates, hat hasten degradation of existing amyloid deposits e.g., immunotherapy or that disrupt the interaction between amyloidogenic proteins and accessory molecules, such as heparan sulfate proteoglycans [1-9].

## Conclusion

Although not common, amyloidosis is a serious disease that results in significant mortality and morbidity. The most challenging issue is significant delay in diagnosis, sometimes up to several years. Early suspicion and thorough investigation are critical to making a timely diagnosis and referral for treatment.

### Take Home Points

- Amyloidosis refers to extracellular, eosinophilic, amorphous Congo Red positive tissue deposition of fibrils composed of low molecular weight subunits of a variety of proteins, derived, in turn, from soluble precursors which undergo conformational changes that lead to the adoption of a predominantly antiparallel beta-pleated sheet configuration.
- The two major forms of amyloidosis are the AL (primary) and AA (secondary) types. AL amyloid, the most common form in developed countries where as AA amyloid is commoner developing countries like ours.
- Some clinical and laboratory features that suggest amyloidosis include waxy skin and easy bruising, enlarged muscles (e.g., tongue, deltoids), symptoms and signs of heart failure, cardiac conduction abnormalities, hepatomegaly, evidence of heavy proteinuria or the nephrotic syndrome, peripheral and/or autonomic neuropathy, and impaired coagulation.
- Tissue biopsy with Congo red staining demonstrating apple green birefringence under polarizing microscopy should be used to confirm the diagnosis in all cases of amyloidosis. Fat pad aspiration biopsy is less likely than liver, renal, or even rectal biopsy to be complicated by serious bleeding.
- The presence of a monoclonal protein alone is not sufficient to make a diagnosis of AL amyloid in a patient with documented amyloidosis unless immunofluorescence has demonstrated monoclonal light chains in the amyloid deposits.
- Heritable types of amyloidosis should be excluded if a plasma cell dyscrasia MM or WM cannot be documented.
- Treatment of the different types of amyloidosis generally varies with the cause of fibril production. A number of novel approaches to treatment are being investigated through *in vitro* drug screening and in animal models, and some have progressed through phase 2/3 clinical trials.

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