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Chapter

Diagnosis of Amyloidosis: Clinicopathological Advances and Challenges

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Abstract

Amyloidosis is a systemic disease with different subtypes of misfolded, insoluble proteins, which are deposited in viscera and thereby cause damage to the affected organs. Its clinical manifestations are highly variable depending on the organs and tissue affected and often require a high degree of clinical suspicion to achieve correct diagnosis. Development of proteomic tools, radioisotope scintigraphy, immunologic antibody targets by immunohistochemistry, immunofluorescence and microscopic techniques have improved the sensitivity of accurate detection of specific subtypes of amyloid proteins. Newer therapeutic targets have been developed to arrest or suppress the specific types of amyloid proteins, giving rise to the possibility of targeted therapy with better quality of life and improved survival benefits for patients. AL, ATTR, AA and ALECT2 are the major subtypes of amyloidosis and kidney, heart, soft tissue, and peripheral nerves are the most affected viscera. The key to achieving success in better quality of life and overall survival in patients, is in early diagnosis and accurate subtyping of amyloidosis.

Keywords: amyloidosis, early diagnosis, amyloid subtyping

1. Introduction

Amyloidosis refers to tissue deposition of extracellular proteins which are misfolded aggregates, characteristically highlighted by apple green birefringence when stained with Congo-red stain and visualized with polarized light in tissue sections [1–3]. The gold standard for diagnosis of amyloid deposit is however by demonstrating the cross beta pleated appearance on X-ray spectroscopy and more recently by proteomic techniques. However, with advances in spectroscopic techniques and nuclear magnetic resonance, a lot of structural details and variations have been described on the parallel cross-beta pleated structure of amyloid proteins and its various subtypes [2].

Amyloidosis causes symptoms and visceral damage by occupying the space in the extracellular region, altering the visceral architecture, and compromising the blood supply to the specialized visceral parenchymal cells. Better techniques have elucidated the cytotoxic effects of the amyloid proteins in addition to the pathogenetic effects of extracellular space occupying mass lesion of amyloid [2]. More than 30 different

types of amyloid proteins have been identified currently. These amyloidosis subtypes display widely variable clinical manifestations, heterogeneous organ involvement, and diverse rate of progressive visceral damage. The need for accurate diagnosis of amyloidosis subtypes reflects these considerations. There is a significant difference in the subtypes of amyloidosis affecting patients from different geographic regions. In Europe and America, amyloid light chain (AL) type of amyloid is the most common subtype, preferentially affecting older adults. Kidney is the most affected viscera in the AL type amyloidosis with heavy proteinuria as a common manifestation and renal biopsies showing markedly divergent deposits of amyloid in glomeruli, tubular basement membrane, interstitium and blood vessels. Immunofluorescent evaluation is invariably one of the earliest identifiers of light chain restriction of either kappa or lambda light chains in the glomeruli and blood vessels being the most common sites of deposition. The findings are corroborated and confirmed on transmission electron microscopy. Type AA amyloid is much more commonly reported in tropical countries secondary to commonly prevalent infectious diseases with resultant inflammation and the management of the disease rests on correct diagnosis and treatment of the underlying cause of inflammation. Immunohistochemistry in tissue sections or cytology aspirates has high sensitivity for identification of AA amyloid. Transthyretin amyloid is commonly detected in patients with cardiac amyloidosis, clinically manifested even in early stage by slow wave changes in electrocardiography and echocardiography findings for restrictive cardiomyopathy [4].

Detection of amyloid protein has variable sensitivity in tissue sections and multiple variables affect its sensitivity such as the density of the amyloid deposits, thickness of the tissue section, quality of the histochemical staining, microscope illumination and quality of the polarizer. Examination of the congophilia tissue under Texas red filter in fluorescence microscope offers much higher diagnostic sensitivity and specificity than that of polarized light. The importance of detecting amyloid early and in smaller quantities is essentially to diagnose the condition early, so that necessary treatment can be administered to stabilize and arrest the progression of end-organ damage [5].

Critically, the management of amyloidosis is significantly different for various types of amyloid proteins, and it has become imperative to exactly subtype the amyloidosis for a targeted therapy as well as a guidance for determining its underlying cause [6].

2. Diagnostic approach of amyloidosis

Amyloidosis presents either as a localized disease or systemic manifestations. Most commonly skin, larynx and bladder are the common sites of localized deposition of amyloid. In several instances, specific organs such as kidney, heart, or liver are affected without involvement of other organs. Having a high index of clinical suspicion leading to appropriate investigations before manifestation of overt symptoms is the key to early diagnosis.

For example, neuropathy involving autonomous or peripheral nervous system is a common but early and non-specific manifestation of different types of amyloidosis. These may include divergent autonomous, sensory, or motor dysfunction of the limbs; gastro-intestinal tract symptoms (diarrhea and obstruction) and urogenital tract (erectile dysfunction); or postural hypotension [1].

Once amyloid deposit is proven in tissue or cytology smears, further study with radiolabelled I123 administration and detection with radionuclide scintigraphy may help evaluate the distribution of amyloid in different organ systems and stage the extent of the disease. However, this diagnostic facility is not available in many centres and serum amyloid protein (SAP) scintigraphy studies are much more commonly used.

It is also important to know the correct clinical context in deciding the diagnostic workup, particularly at an early stage. Secondary amyloidosis type AA should be high in the clinical suspicion in a patient with long standing infections such as tuberculosis or chronic inflammatory conditions such as in Crohn's disease or auto-immune conditions with long history of remission and relapses. Primary amyloidosis is most often sporadic but can be rarely familial. Among many causes of sporadic amyloidosis, AL type amyloidosis due to tissue deposition of amyloidogenic monoclonal light chains is most frequent and is often manifested by proteinuria. A high index of suspicion, leading to a kidney biopsy for tissue identification of amyloid deposition composed of monoclonal light chain revealed by IF is essential for diagnosis. It is also important to understand that light chain restriction alone is not diagnostic of AL amyloidosis. Identification in light microscopy (LM) of a pale homogeneous eosinophilic deposit, particularly localized to glomerular mesangium or blood vessels is the key to prompt further work up with Congo-red staining followed by examination under polarized light complemented with Texas Red immunofluorescence. (**Figure 1**) Texas Red immunofluorescence has significantly increased the sensitivity and specificity of detection of amyloid in kidney biopsy. Also, electron microscopy helps in identification of amyloid deposits in tissue samples where the deposits are in tiny quantities or with immune-gold electron microscopy, in situations where monoclonal gammopathy of undetermined significance or hereditary amyloidosis with coexisting AA type amyloid needs to be excluded [1].

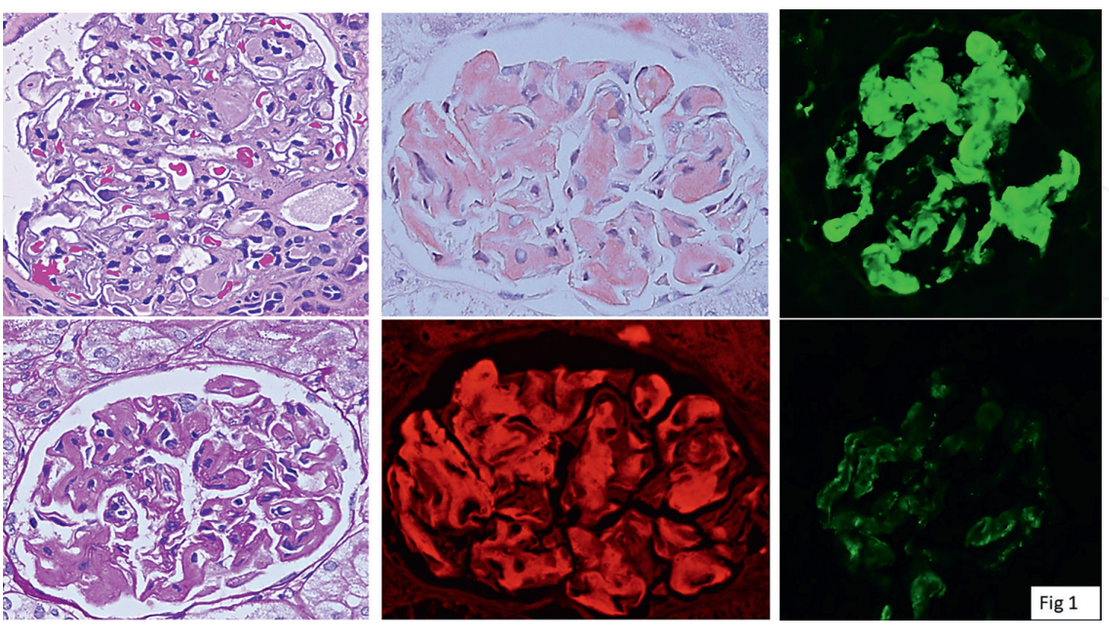

Figure 1.
Sections highlight glomeruli with pale homogeneous eosinophilic deposit in the mesangial region which has a pale PAS staining, congophilia highlighted by Texas red immunofluorescence filter. The section showed strong kappa restriction on immunofluorescence stain and lambda was negative.

Testing for amyloid is very often performed in "surrogate" tissues such as abdominal fat pad, tongue, or rectal biopsies. The main reason is the easier accessibility and possibility of repeat testing when warranted. The draw-back of this approach is the limited sensitivity, when compared with testing in a potentially affected organ. For example, the chance to diagnose AL amyloidosis is much higher in a kidney biopsy than in surrogate tissue, reflecting preferential renal deposition of amyloidogenic light chains even at a very low blood level. Detection of amyloid deposits would be quite unlikely in "surrogate" tissue such as fat pad at such a low circulating blood levels of amyloidogenic protein [7].

Within the wide scope of the diagnostic approach for cardiac amyloidosis detailed elsewhere, cardiac amyloidosis is essentially detected by identifying low voltage QRS changes and a thick-walled non-dilated left ventricle in echocardiography. Serum marker studies for troponin proteins and N-terminal brain natriuretic peptide (NT-proBNP) are extremely useful markers in screening, detection and prognostication of patients with cardiac amyloidosis. These are helpful essentially in view of the minimally invasive nature of these investigations and the ability to quantify serum levels of these markers with validated techniques. The role of cardiac magnetic resonance (CMR) in the diagnosis of amyloid is still in evolution. Bone avid radioisotope scan and technetium scintigraphy is a highly sensitive and specific method of detection of cardiomyopathy of ATTR type, in the absence of cardiac biopsy, in patients without light chain restriction as defined by work-up with free light chain assay (FLC) and negative serum and urine immunofixation electrophoresis. This is because, AL light chains in myocardium may result in false positive tests. Since AL cardiac amyloidosis cannot be distinguished from wild type transthyretin (senile type) the use of technetium pyrophosphate scan to distinguish ATTR from AL becomes critical. However, increasingly immunoglobulin abnormalities are being

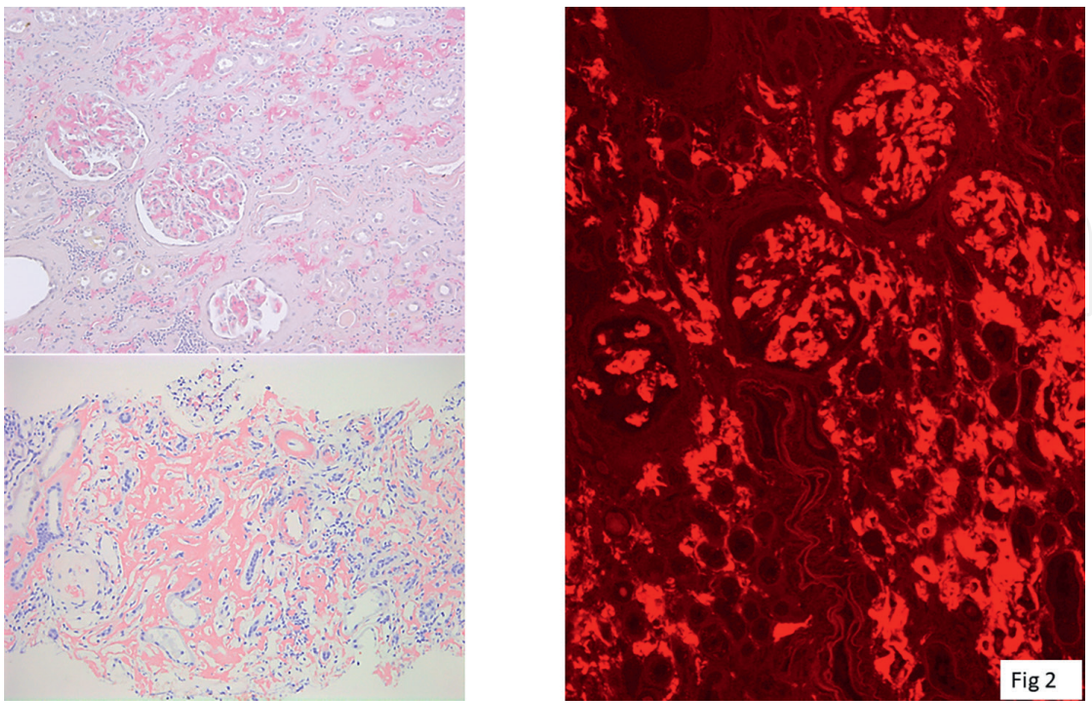

Figure 2.
Sections show prominent interstitial and vascular deposits of amyloid in ALECT2 type of amyloidosis with congophilia, highlighted by Texas red immunofluorescence filter.

detected with increasing frequency in ATTR amyloidosis and in such situations, tissue for mass spectroscopic analysis is the diagnostic standard to avoid errors [7]. Each of these diagnostic modalities has its pitfalls and variable utility among different types of amyloidosis. For example, low QRS voltage in EKG is more frequently observed in AL type amyloidosis and much less commonly in ATTR type [4].

While we emphasize on the high index of clinical suspicion for evaluation of amyloidosis, it is important to remember that clinical symptoms can be highly variable. For example, though proteinuria of sub-nephrotic or nephrotic range is the most common presentation of renal amyloidosis, newly identified ALECT2 amyloidosis, is known to present with bland urine sediments in the absence of or minimal proteinuria, in a setting of hypertension and chronic renal failure. This subtype shows extensive interstitial deposits in the kidney compared to deposits in the glomeruli. (**Figure 2**) Though this subtype was initially reported much more commonly in Hispanic population in North America, better awareness and availability of diagnostic methods have helped in identification of ALECT2 amyloidosis in different geographic regions in the world [8].

3. Stains used in demonstration of amyloid in tissue sections

Amyloid was initially identified as a type of cellulose by Rudolf Virchow, who observed the color change from brown to blue on slices of an amyloid containing spleen exposed to sulfuric acid followed by iodine [9]. Subsequently various metachromatic stains were identified to be useful in detection of amyloid. With better advances in X-ray crystallography, it was established that amyloid is a protein product with structural characteristics. Congo red is the most commonly and popularly used histochemical stain and the characteristic apple green birefringence under polarized light is linked to the cross beta pleated structure of the fibrils. Other traditional stains which are not very commonly used now are iodine-sulfuric acid, methyl violet, crystal violet, Nile red. Thioflavin T or S has high sensitivity in detection of amyloid in tissue. Thioflavin T is popular, in view of its higher sensitivity and the ability to quantify the amyloid protein detected by this stain [10].

Detection of pale, homogeneous eosinophilic material with no evidence of nuclei amidst the material, seen in tissue sections stained in hematoxylin and eosin raises the suspicion of amyloidosis. In particular, the deposition is focused on certain sites such as blood vessels of different sizes, superficial dermis, hepatic sinusoids (**Figure 3**), and interstitium of alveolar spaces etc. Other histochemical stains such as periodic acid-Schiff (PAS) and silver stains are generally negative or display only pale staining. (**Figure 4**) Under Masson's trichrome stain, amyloid appears pale blue or gray. These features are helpful in deciding appropriate sections and foci for Congo red or thioflavin staining. Another important feature is that congophilia, which is indicative of the Salmon pink colored stain in the tissue site of interest, is not specific for amyloid.

In addition to the usual observation of glomerular mesangial and vascular deposits which are homogeneous, acellular in hematoxylin and eosin and pale stained in PAS and silver stains, peculiar spiculated tubular casts have been identified in renal biopsies with a particular lamellated appearance exhibiting the characteristics of fractured tubular casts evoking histiocytic reaction and apple green birefringence in polarized light when stained with Congo red stain. These fractured lamellated tubular casts of amyloidosis are however strongly PAS and silver positive. Though such observations are in few individual case reports, it is interesting as these have been observed in

patients with no glomerular deposits and requires high index of suspicion to evaluate further for amyloidosis in this setting in the absence of glomerular and vascular deposits (**Figure 5**) [11].

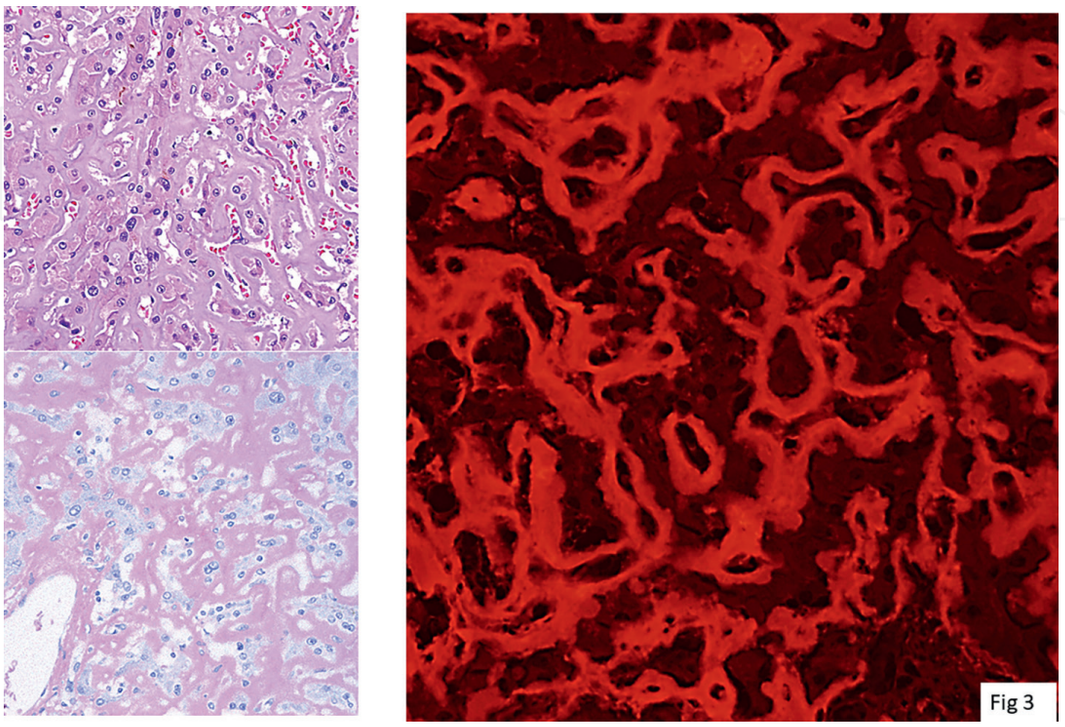

Figure 3.
Sections show prominent sinusoidal deposits of homogeneous eosinophilic material which is pale in PAS stain and exhibiting strong fluorescence under Texas red immunofluorescence.

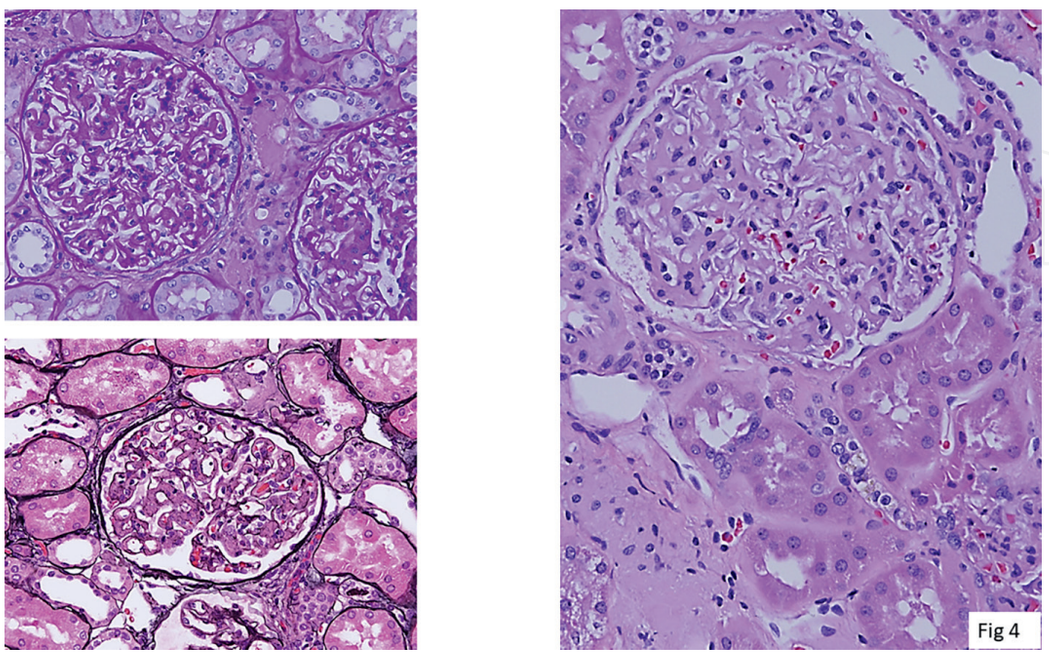

Figure 4.
Sections show the staining characteristics of amyloid by hematoxylin and eosin stain which shows pale acellular matrix which is pale in PAS stain and negative for silver stain.

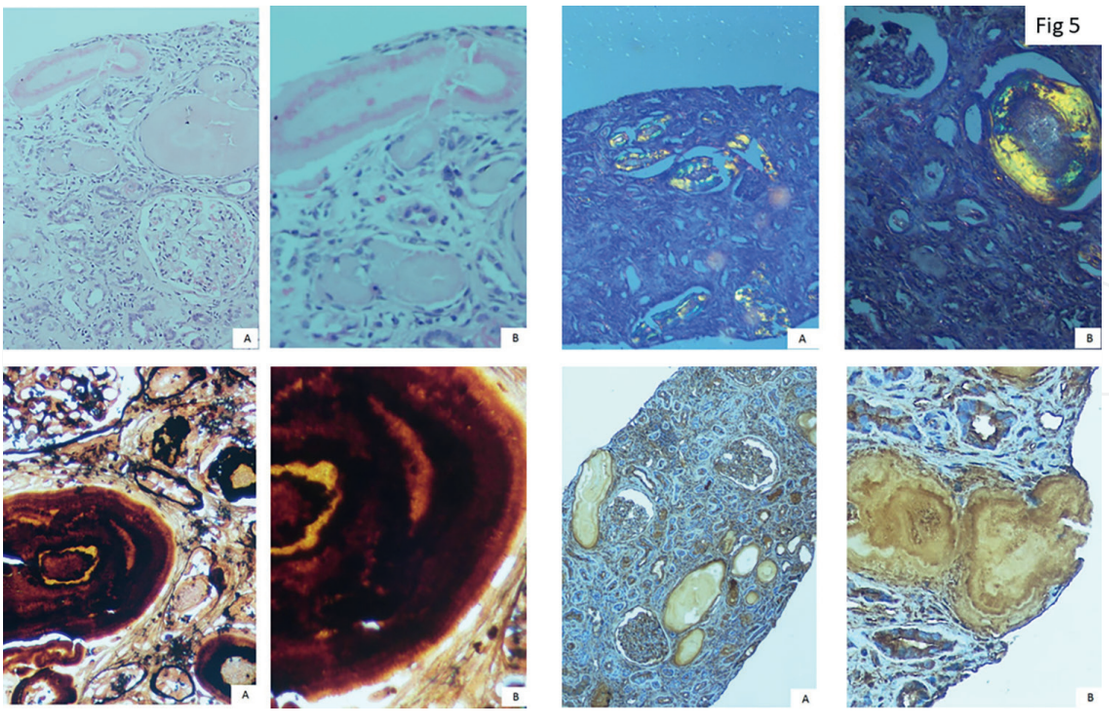

Figure 5.
Section shows lambda light chain restricted amyloidosis (by immunohistochemistry) confined to the tubular casts showing the prominent lamellated appearance in hematoxylin and eosin and silver stains with apple green birefringence under polarized light.

4. Points of critical importance in amyloid detection in tissue

Tissue or cytology cell block sections for detecting amyloid must be thicker (6–10 μm) than the tissue sections for routine histology (3–4 μm) The purpose of the thicker section is to have a higher concentration of amyloid so that the histochemical stains, either Congo red or thioflavin T would be able to react and elicit the positive reaction. In addition, in cytology aspirates, it would be good to observe the presence of arterioles, as amyloid proteins tend to have predilection for these vessels.

Examination under a good quality microscope, with stronger light illumination is a critical requirement for detection of birefringence under polarized light. Accurate identification of amyloid in tissue sections is also critical in case where there is a need to dissect the amyloid containing tissue portions, which can be minuscule, out of the tissue block for further electron microscopy study or for mass spectrometric classification of amyloid types.

5. False positive and false negative tests with Congo-red staining

Congo red is a tricky stain to perform and interpret. The Congo red stain can be often falsely positive or negative. The false positive result reflects non-specific binding of Congo red dye molecules to non-amyloid proteins in tissue sections. Several technical modifications of the original Congo red stain have been developed to attenuate this nonspecific binding, but they do not help to completely eliminate this problem. A specific binding of Congo red dye molecules and amyloid fibrils regardless of chemical type creates a distinctive chemical structural configuration that appear “apple-green”

under polarized light, leading to the repeatedly quoted dictum that the diagnosis of amyloidosis requires not only a positive Congo red stain but also an associated apple green birefringence. Recent findings may detract from this dual requirement. As mentioned above, several technical conditions are required for an observable apple green refringence even in tissue sections with bona fide amyloid deposition. These conditions are often not met. Recent international consensus indicates that under polarized light, Congo red-positive amyloid can display not only apple-green, but also other colors including yellow or brown, and yet some of these colors are also observed for Congo-red positive non-amyloid tissue, such as collagen or fibrous tissue [12, 13].

Along the on-going attempt to improve the diagnostic utility of the Congo red stain, recent studies suggest that examination of the congophilia by immunofluorescence scope with a Texas Red filter would significantly improve both the diagnostic sensitivity and specificity of the Congo red stain [5].

6. Importance of genetic testing in amyloidosis

Familial type of amyloidosis, commonly detected in the familial type of transthyretin amyloid (ATTR) of cardiac amyloidosis, needs genetic work up for diagnosis. The wild type or sporadic type of ATTR is seen mostly in the elderly, reflecting age-related changes of the heart, while the familial ATTR affects younger age group. Also, several newer forms for hereditary amyloidosis have been identified with better proteomic techniques and mass spectrometry. For example, alpha chain of fibrinogen A (AFib), Apolipoprotein AI and AII, lysozyme (Alys) and gelsolin are examples of hereditary amyloidosis and genetic studies are warranted in these subtypes to identify and characterize the risk for the patient and among other family members.

Hereditary amyloidosis may present in patients without a definitive family history. This is since most forms of hereditary amyloidosis have an autosomal dominant inheritance and incomplete penetrance and expressivity, accounting for highly variable clinical manifestations. However, for some form of hereditary amyloidosis such as the AFib type, mass spectrometry can identify the chemical type, and furthermore recognize chemico-structural motifs that suggest a hereditary variant for further genetic confirmation [14, 15].

7. Role of immunofluorescence in diagnostic work-up of amyloidosis

The single most important role for immunofluorescence (IF) in amyloidosis work-up is for identifying light chain (AL) amyloidosis in kidney biopsies. The importance of this detection cannot be over-emphasized, as this is invariably the earliest detection of clonal restriction of light chains resulting in further work-up with serum and urine for immunofixation electrophoresis and subsequently other screening methods. IF study is traditionally performed only in fresh frozen tissue. Since kidney biopsies are the only type of tissue sample being routinely submitted in fresh frozen state in addition to routine histology, the diagnostic utility of IF for diagnosing and typing of amyloidosis, is virtually limited to this type of tissue samples (**Figure 6**) [1].

Thioflavin T is a fluorescent dye, thus requiring IF examination for amyloid detection [10]. Examination of Congo-red stain using Texas Red filter, increases the diagnostic sensitivity and specificity of this stain. This is another utilization of immunofluorescence in tissue examination for amyloid [5].

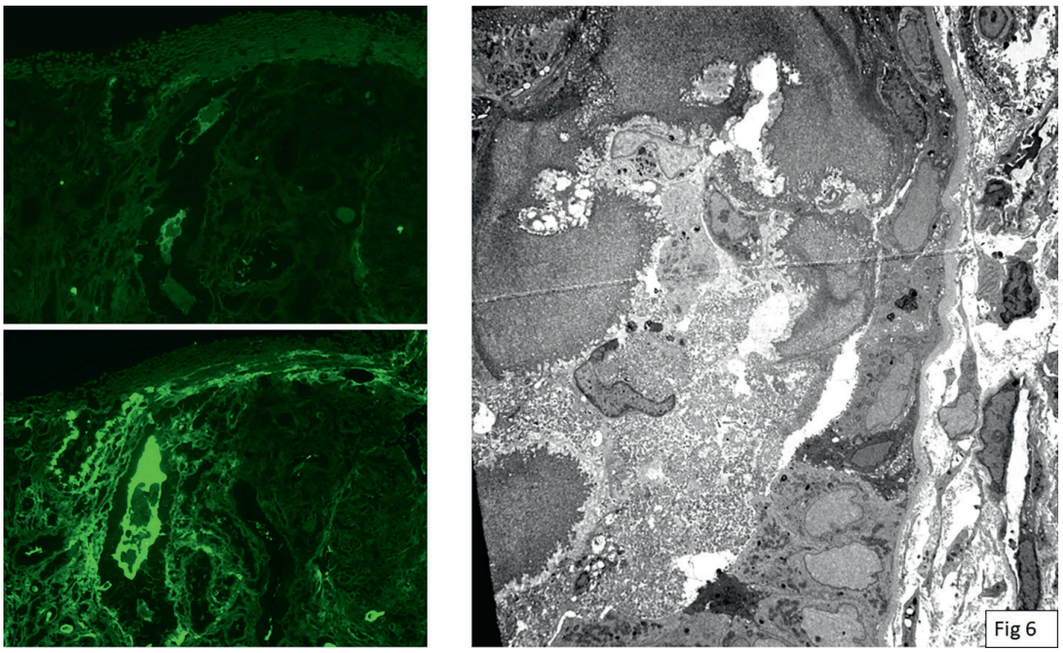

Figure 6.
Sections show lambda light chain restriction in tubular casts by immunofluorescence, and amyloid deposit from the cast is highlighted by randomly arranged fibrils in the tubular ultrastructure.

8. Role of serum and urine light chain measurements

In those patients with high index of clinical suspicion favoring diagnosis of a light chain-restricted amyloidosis, such as rare patients presenting with macroglossia in absence of light chain restriction in biopsy or those with light chain restriction in kidney biopsies on immunofluorescence examination, patients are further assessed for light chain restriction by serum and urine electrophoresis. The circulating light chains may be present in very low quantities, which cannot be detected by routine electrophoresis, but are obvious in immunofixation electrophoresis.

Serum free light chain assay (sFLC), has much higher sensitivity than serum and urine immunofixation electrophoresis, reaching a level of 10-fold more. However, the very high sensitivity limits its independent use. The normal serum κ:λ ratio by FLC assay is 0.26 to 1.65, and it is important to emphasize that since the light chains are filtered through the glomerulus, the κ:λ ratio is more critical than the absolute levels. It is also important that patients with renal disease and elderly patients are at higher risk of showing elevated free light chain levels, when studied with highly sensitive methods. The advantage of sFLC assay is that being a highly sensitive quantitative assay, it can be effectively utilized in identifying the light chain restriction at the baseline and monitor the treatment effectiveness on follow up [6, 16].

9. Role of immunohistochemistry in diagnostic work-up of amyloidosis

Immunohistochemistry staining performed in formalin fixed paraffin embedded tissue sections is most useful in staining for type AA amyloid to detect secondary amyloidosis. It is particularly useful, as identifying the exact cause of secondary amyloidosis is not always straight-forward, as there may be several confounding diseases and factors influencing the condition in each patient. Also, detection of

newer subtypes of amyloid such as ALECT2, AFibr is significantly made easier with availability of immunohistochemical antibodies for their staining.

Amyloid is a sticky protein, which can trap other proteins and cause non-specific positivity. Unlike immunofluorescence, immunohistochemistry has limited sensitivity for detection of light chain restricted AL type amyloidosis. This is due to several factors such as fragmented nature of light chains in amyloidosis, variations in antigen extraction techniques and difficulties in designing primary antibodies in immunohistochemistry for the different types of restricted light chains. Although lambda light chain is the most common subtype that is restricted in AL amyloidosis, restriction of kappa light chain, and rarely even heavy chain restrictions or combined heavy and light chain- restricted amyloidosis have all been reported. This explains the challenges and limitations of using immunohistochemistry in diagnosis of AL amyloidosis [8, 14, 15].

Immunofluorescence and immunohistochemistry will remain major diagnostic tools in basic subtyping of amyloidosis, due to their wider availability, better standardization, and reporting in most hospital-based pathology laboratories.

10. Role of electron microscopy in diagnostic work-up of amyloidosis

Transmission electron microscopy is a useful diagnostic tool in the armamentarium, particularly in the evaluation of kidney biopsies. This is critical in the setting of glomerular deposition diseases, where the mesangial region shows ill or well-formed nodules due to deposits of various organized deposits. Electron microscopy is useful in the work-up of differential diagnosis in this setting, with detection of randomly oriented fibrils of around 10 μm diameter. (**Figure 7**) However, few types of amyloid

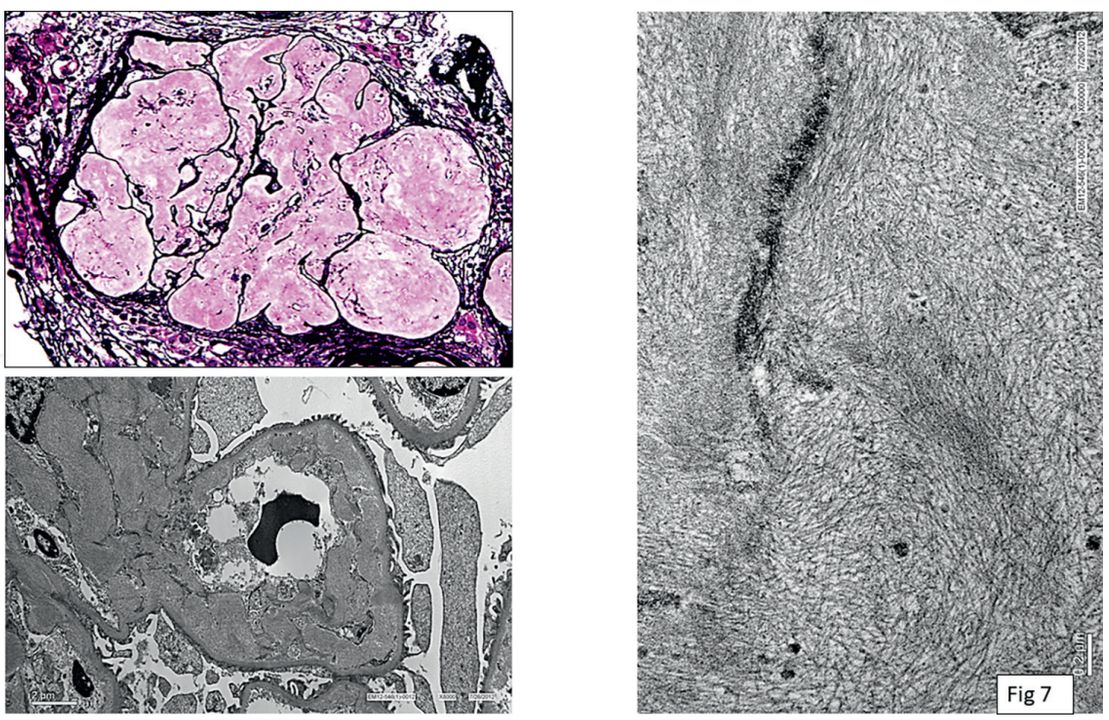

Figure 7.
Sections highlight the prominent PAS and silver negative amorphous acellular amyloid deposits in the glomerulus, which is highlighted in the subendothelial and mesangial region of the glomerulus in transmission electron microscope. Higher magnification reveals the characteristic randomly arranged fibrils in 10 μm range.

may have thicker fibrils. It has also been described that aggregation of ATTR amyloid protein occurs in association with collagen IV, fibronectin, and laminin. In addition to kidneys, electron microscopy has also been widely utilized in studying the characteristics of different types of amyloid in various organ systems in heart, peripheral nerves, brain, liver, spleen etc.

Ultrastructural examination facilitates the distinction of amyloid fibrils from other types of fibrils such as those in diabetes or much larger fibrillary deposits in fibrillary or immunotactoid glomerulonephritis. However, it cannot effectively distinguish different subtypes of amyloid. Immunogold-electron microscopy can be utilized for subtyping, but due to complex procedural requirements, it is not a popular diagnostic method and is mostly confined to research settings [1, 2, 17].

11. Role of tandem mass spectrometry in subtyping of amyloid proteins

After amyloidosis is identified, determining its chemical type, each of which may have a distinctive clinicopathologic profile, is essential for management and prognostication. Immunohistochemical staining using amyloid protein type-specific antibodies can be used for this goal. However, this approach is limited by several technical considerations including the need to maintain a large battery of infrequently utilized antibodies.

Laser capture and mass spectrometry-based proteomics is now the most optimal approach. This procedure starts with laser capture micro-dissection from tissue block foci of amyloid deposits detected by a Congo red stain, followed by mass spectrometry study of the capture protein. Since each chemical type of amyloidosis yields a specific and distinctive mass spectrometric pattern, chemical typing of amyloidosis is possible. Although this technique is available only in specialized Institutions, it currently represents a standard clinical care test, rather than a research tool. In addition to represent an essential clinical utility, this technique has also helped to detect several novel types of amyloidosis [1, 2, 6, 18].

12. Other serological assays to screen for amyloidosis

Measurement of serum amyloid A, a precursor of tissue AA amyloid, or C-reactive protein is used in screening and detection of secondary and hereditary amyloidosis. Elevated serum amyloid A levels in correlation with tissue deposits of AA type amyloid can be taken as corroborative evidence for diagnosis of secondary amyloidosis. CRP is an acute phase reactant and though easily available as a sensitive screening marker, it is not specific for amyloidosis.

Serum alkaline phosphatase levels are elevated in patients with amyloidosis of liver, and it is useful in a setting where transaminase levels are normal. This is a considered a characteristic feature of hepatic amyloidosis as the amyloid deposition is in the sinusoids [1].

13. Prognostic markers in amyloidosis

Various new prognostic markers have been identified for following patients under treatment and for identifying progression of the disease. The markers are dependent

on the involved viscera. Measurement of urine proteins, if possible, a 24-hour quantification is a useful tool, particularly considering that renal involvement is one of the commonest manifestations of amyloidosis.

Measurements of serum amyloid protein A and C-reactive protein as acute phase reactant can be useful.

Serum free light chain measurement is useful in monitoring the disease progression in patients with AL amyloidosis [6].

Similarly, levels of serum albumin and alkaline phosphatase can be used to monitor the progression of the disease in appropriate settings [1].

Efforts have been made to quantify thioflavin T fluorescence in tissue sections at specific wavelengths to try and assess the aggregation kinetics and its correlation with disease progression. Thioflavin T concentration at 10–50 μm gives maximum sensitivity for aggregation kinetics. However, this is not widely available in diagnostic practice as these results need to be validated in multi-centric studies. Also, the standardization of quantification settings across various laboratories will be a requirement for its use in patient care setting [10].

Serum levels of cardiac troponins and NT-proBNP are very useful not only in initial screening but also in the monitoring of damage to cardiac myocytes, stage and assess the progression of the disease [1, 6].

The type of light chain restriction in AL amyloidosis has been studied for its variation in organ involvement, progression free and overall survival. It is interesting that there is a significant difference between those with λ light chain type amyloidosis, who present predominantly with renal and neurological manifestations while patients with κ restricted AL amyloidosis had liver disease more commonly. Though with stem cell transplantation, both the subtypes showed similar complete and overall response rates, patients with κ-AL amyloidosis had better progression free and overall survival and this result was validated by multi-variate regression to be an independent predictor of survival [6].

Even among patients with lambda light chain amyloidosis, germline differences have been studied with mRNA sequencing to identify variations in organ involvement and disease progression. The subtype IGLV3 was the most frequently detected in patients with cardiac disease, while IGLV1 was common in patients with renal involvement. IGLV6 subtype was the most common in patients with combined cardiac and renal disease. On further detailed analysis, IGLV3-21 was the most common in cardiac disease, while IGLV1-44 was common in renal AL amyloidosis. In the combined group with cardiac and renal disease, IGLV6-57 was the common subtype [18].

14. Brief note on specific rare subtypes of amyloidosis

It will be beyond the scope of this chapter to discuss all the rare subtypes of amyloidosis. However, few important variants of amyloidosis are discussed below, in view of their clinical and diagnostic significance.

15. Aβ amyloid in neurodegenerative diseases

Aβ amyloid, is derived from amyloid precursor protein (APP), a normal metabolic product of the cellular metabolism, synthesized by the endoplasmic reticulum and subsequently processed in the Golgi apparatus. The APP protein undergoes

maturation in the Golgi apparatus and the mature form is present in the plasma membrane. In the plasma membrane it is actively cleaved either by the non-amyloidogenic pathway by α-secretases followed by γ-secretases, or by the amyloidogenic pathway by enzymes β-secretase and subsequently by γ-secretase. The cleavage of APP by the amyloidogenic pathway results in abnormal accumulation of Aβ amyloid in the hippocampus and other regions of the brain which are critical for memory and cognitive function, resulting in neuro-degenerative conditions particularly Alzheimer's disease or parkinsonism. Aβ oligomers are water-soluble and dissipates diffusely in the brain, but these are crystallized and stabilized by various interactions with metals such as Cu, Zn, Fe etc. resulting in large aggregates of insoluble amyloid plaques. The exact structural detail and behavior Aβ oligomers are not yet fully understood. Several constitutive activation mechanisms are at play resulting in aggregation of insoluble amyloid plaques, causing disease progression. Significant advances in solid state nuclear magnetic resonance spectroscopy, have highlighted variations in the cross beta pleated structure of this amyloid protein. Site directed spin labelling and electron paramagnetic resonance (SDSLEPR) spectroscopy have helped in confirming the ultra-structural detail of the aggregated amyloid fibril complex and its conserved nature.

The insoluble large aggregates of Aβ amyloid were identified initially in patients with neuro-degenerative diseases and were thought to be neurotoxic causing cell death, memory, and cognitive loss. However, with improvements in technology, it is now clear that Aβ oligomers or the pre-fibrillar Aβ proteins are much more neuro toxic. Soluble Aβ binds to several molecules in the cell membrane, receptors as well as targets in extracellular space. Binding of ApoE to Aβ helps in receptor mediated endocytosis and cellular uptake of Aβ, as well as through vesicular transport system.

The formation of Aβ proteins and its stabilization is counteracted by proteolytic degradation by several enzymatic pathways. These degradation pathways have been studied in detail and have found their significance in the pathogenesis of neuro-degenerative conditions. However, the exact role of each of the enzymes in the pathogenesis is still poorly understood.

Though Aβ protein aggregation is not the only etio-pathogenetic cause for Alzheimer's disease or several other degenerative diseases, improved understanding has significantly helped us to understand the disease biology. Greater understanding of the structural and biochemical properties of Aβ proteins and their aggregation have helped in identifying several new compounds that can act by blocking the aggregation or stabilization of Aβ proteins, increased degradation of these proteins, or using immune mediated targets such as immunization. Several compounds used in oncology such as immune check point inhibitors, PD1/PDL1 and anti-diabetic medications have also been studied in the therapeutics of neuro-degenerative diseases [2].

16. Leukocyte chemotactic factor 2 amyloidosis (ALECT2)

It is a recently described subtype of amyloidosis, detected predominantly in Hispanic population in autopsy studies. This subtype commonly affects kidney, liver and lungs while involvement of the myocardium has not yet been described. This subtype of amyloid is clinically important since this causes gradually progressive chronic renal failure with bland urine sediments. Also, ALECT2 amyloid has not been found in subcutaneous adipose tissue or salivary glands. So far, no serological markers are available for the diagnosis of LECT2 amyloid. Thus, this makes the possibility of

screening through surrogate biopsies impossible for this type of amyloidosis. The only effective method to diagnose ALECT2 amyloidosis is by the biopsy of the kidney or liver and staining for anti-LECT2 by immunohistochemical antibodies or mass spectrometry typing. However, in view of the clinical presentation the patients will not fulfill the clinical indications for biopsy of the viscera. This makes it challenging and commonly, the diagnosis is made as incidental finding or when significant damage has already occurred in the kidney and other viscera. This is not an uncommon cause of chronic renal failure at least in the Hispanic population and needs further evaluation. It is also important that this needs to be studied extensively in other populations, as it is most likely underdiagnosed due to the lack of specific testing.

Another interesting observation is that the deposits of ALECT2 amyloid in the kidney is predominantly in the interstitium, rather than formation of mesangial nodules in glomeruli. In the liver, portal tract, lobules and arteries are the common sites while red pulp of the spleen shows deposits of ALECT2 amyloid. ALECT2 amyloid has not yet been identified in brain and fibro-adipose tissue so far.

ALECT2 amyloidosis can co-exist with other common subtypes of amyloidosis such as AL or AA and needs to be specifically evaluated to avoid underdiagnosis. ALECT2 should not be incorrectly diagnosed in patients with other types of amyloidosis to avoid toxicity of inadvertent treatment. ALECT2 amyloidosis is not a genetic disease since patients maintain a wild-type genotype and mutations of the leukocyte chemotactic gene has not been identified. Thus, genetic screening of family members is not an effective diagnostic strategy [8, 15].

17. Fibrinogen Aα type amyloidosis (AFibr)

This is a relatively common hereditary type of amyloidosis with autosomal dominant inheritance and renal manifestation with proteinuria. However, a significant percentage of these patients do not carry a family history of amyloidosis due to the reduced penetrance and variable expressivity of the disease. DNA sequencing has helped in identifying the common mutations associated with AFibr type of amyloidosis. Kidney biopsy characteristically reveals enlarged glomeruli with abundant mesangial deposits of AFibr amyloid and Immunohistochemistry is highly sensitive for detection of this subtype in renal biopsies. It is important to recognize this subtype, as unlike AL amyloidosis, AFibr type has an indolent clinical course and has good outcome post renal transplant with no reports of recurrence. However, no definitive treatment is available and management strategy is essentially supportive [14].

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