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Chapter

Pathologic Findings of Amyloidosis: Recent Advances

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Abstract

Amyloids are aggregations of misfolded protein, which creates fibrillary structures. Unlike normally folded proteins, misfolded fibrils are insoluble and deposited extracellularly or intracellularly. The pathologic mechanism is still unclear, but resultant toxic oligomers within the tissue are known to damage the tissue via aberrant protein interactions. This condition has been known as amyloidosis. Different kinds of amyloid protein may cause similar or different clinical signs and symptoms, largely depending on the target organ it is deposited. However, because treatments and prognoses of each type are different drastically, it is critical to distinguish them and determine the specific type of amyloidosis. The confirmation and typing of amyloid heavily depend on pathologic examination of tissue. The gold standard method for the former is a Congo red staining and birefringence under polarized microscopy. The conventional way for the latter is immunohistochemistry (IHC), where most of the amyloid types can be classified. However, electron microscopy, mass spectrometry, or other molecular methods are required for typing some amyloids that are difficult to identify through IHC. In this chapter, we will describe basic concepts of amyloidosis and pathologic findings of amyloid deposition, including atypical structural deposition. Furthermore, we will review methodologies for amyloid typing briefly.

Keywords: amyloid, amyloidosis, immunohistochemistry, molecular diagnosis, immunoelectron microscopy, atypical amyloid feature

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1. Introduction

Amyloids are an aggregation of misfolded protein that creates fibrillary structures for various causes including hereditary or de novo mutations of the proteins and errors in the normal folding processes. This abnormally misfolded protein can aggregate into insoluble polymers or develop resistance against proteolysis. As a result, amyloid is deposited extracellularly or intracellularly within the tissue, causing tissue damage. This condition is called amyloidosis.

More than 90% of amyloid deposition is composed of protein fibrils, and the remainder of the deposition is proteoglycans, glycoproteins, or serum amyloid P components. Because more than 30 previously described species of amyloid protein share the same fibril structure, they may look similar when they are deposited within an organ. The structure of amyloid is observed by electron microscopy showing fibrils with a diameter of approximately 7.5–10 nm. Furthermore, β-pleated sheet structures within the fibrils were shown by X-ray crystallography and infrared spectroscopy.

Depending on the chemical nature and the origin of the amyloid, each amyloid type has a tendency to be deposited in certain tissue or organ. However, the clinical signs and symptoms of different types can be remarkably similar when those types of amyloid are deposited within the same organ. Moreover, the deposited amyloid will show the same bright pink homogeneous amorphous materials when the affected organ is examined microscopically. This can be confirmed by apple-green birefringence on Congo red stain with polarization and fluorescence microscopy. Even if they have a similar morphologic appearance and similar clinical pictures, the treatment and prognosis vary significantly according to the type of amyloid. Thus, distinguishing the amyloid type is essential.

2. Molecular pathogenesis

The mechanisms of amyloidogenesis of each protein are quite variable and involve different overlapping mechanisms and environmental factors. The four recurring themes include intrinsic amyloidogenic tendency, increased concentration, altered proteolytic cleavage, and genetic mutation.

Several indigenous proteins have an innate tendency to fold into amyloid structure. Such proteins include transthyretin (TTR) and atrial natriuretic peptide (ANF). The former can deposit in the heart, joint, and other organs in the elderly, even without genetic mutations, and cause **wild-type TTR amyloidosis (wtATTR)** (formerly, **senile systemic amyloidosis**). In contrary to transthyretin, which is deposited in both the atria and ventricle, ANF deposits selectively in the atria and causes **isolated atrial amyloidosis (IAA)**. This condition is also more common among the elderly and associated with atrial fibrillation. Other intrinsically prone proteins include apolipoproteins A-I, A-IV, and E and serum amyloid protein (SAP), which are incorporated in other forms of amyloid plaques nonspecifically.

Interestingly, exogenous proteins that have an amyloidogenic property can also cause amyloidosis. Two peptide drugs, insulin and enfuvirtide, have recently been described to cause localized amyloidosis [1]. Both drugs are injected subcutaneously, and the drug polypeptides may aggregate into amyloid fibril forming a localized amyloidoma. The amyloid fibrils are composed of the drug peptides themselves, which was confirmed by mass spectrometry. This **pharmaceutical amyloidosis** is an important differential diagnosis in a patient who presents with abdominal nodules and has been on insulin or enfuvirtide therapy.

Another contributing factor is persistently high concentrations of amyloidogenic proteins. Such elevated concentrations make it easier for the proteins to deposit and form a nidus for fibril extension and stabilization. These high levels can be achieved by either overproduction or undersecretion. For instance, SAP expression is greatly increased under inflammatory conditions, where the protein can aggregate to cause **AA amyloidosis**. Another example is **dialysis-associated amyloidosis (Aβ2M)**, where β-2 microglobulin (β2M) level is increased in patients with end-stage renal disease due to ineffective elimination.

Amyloidosis caused by altered proteolytic cleavage is classically exemplified by **Alzheimer's disease**, in which amyloid-β precursor protein (AβPP) forms neuritic plaques. When AβPP is cleaved by β- and γ-secretases rather than normal α- and γ-secretases, a highly amyloidogenic and neurotoxic oligomer Aβ is produced. The Aβ is believed to cause cellular dysfunction and neurodegeneration in **Alzheimer's disease**.

Lastly, genetic mutations of proteins can form amyloid fibrils by one or more mechanisms previously mentioned. These alterations may involve either gene overexpression or structural changes. The former promotes amyloidogenesis by increased concentrations and the latter by either conferring amyloidogenic

instability to the protein or making the protein subject to amyloidogenic proteolytic cleavage. One important example is duplication or triplication of the *SNCA* gene, which results in increased production of the gene product α-synuclein. It aggregates into toxic oligomer and amyloid plaques (Lewy body) and causes familial **Parkinson's disease**. In **hereditary transthyretin amyloidosis (hereditary ATTR)**, more than 130 mutations in *TTR* gene induce further instability to intrinsically unstable transthyretin and result in amyloid deposition in the heart, kidney, and peripheral nerves. Another interesting kind of mutation that can potentially produce amyloid by altered proteolytic cleavage involves gelsolin protein. Several mutations of gelsolin make the protein vulnerable to cleavage by furin, a ubiquitous protein convertase, producing amyloidogenic fragment C68 and causing **hereditary familial amyloidosis of Finnish type**.

Although different mechanisms are shown to be involved in amyloidogenesis, how some amyloid fibrils are deposited selectively in certain organs is not well known. In localized amyloidosis, the location of amyloid deposition may be related to the tissue where the amyloid protein is originated. For instance, islet amyloid polypeptide (IAPP or amylin) is an amyloidogenic peptide with physiologic roles in glucose regulation and secreted along with insulin by Langerhans islet cells. In **type 2 diabetes** and **insulinoma**, IAPP is deposited only in the islet of Langerhans, not exocrine pancreas. Other factors that may explain organotropism of amyloid fibrils include physiochemical environment and extracellular matrix. In **dialysis-associated amyloidosis**, β2m deposition in joint and bone tissue may be explained by the affinity of β2m for collagen, enhanced fibril extension by glycosaminoglycans such as heparan sulfate and bone resorption and nidus formation by proinflammatory cytokines and acidosis.

Another unsettled issue is how amyloid formation can damage the target tissue. Extracellular deposition of amyloid fibrils itself may disrupt the organ integrity as in **cerebral amyloid angiopathy (CAA)**, where Aβ is deposited in the walls of meningeal or cortical vessels, weakens the vessel, and leads to lobar hemorrhage. However, available evidence indicates that the primary mechanism of tissue damage in the majority of amyloidosis involves toxic oligomers rather than mature amyloid fibrils themselves. Despite various cellular defensive mechanisms to prevent proteins from misfolding like molecular chaperones and cochaperones, ubiquitin-protease pathway, and autophagy, such "proteostasis" machineries can be overwhelmed by mechanisms mentioned above. The resultant misfolded oligomers may exhibit hydrophobic residues that are normally buried inside the normal quaternary structure of the protein, and they seem to induce aberrant interactions with other proteins, triggering unfolded protein response, cell death, inflammation, and other pathways of cell injury. Moreover, these toxic oligomers seem to "spread" to the surrounding tissue in a prion-like manner, further propagating cell injury.

3. Major types of amyloidosis

The most recent classification of amyloidosis has been published by the Nomenclature Committee of the International Society of Amyloidosis (ISA) in 2016. The classification listed 36 different extracellular fibril proteins seen in humans and animals, whose sequence is identified unequivocally (**Table 1**). According to this scheme, amyloid proteins can be broadly divided into systemic or localized in relation to the extent of organ involved by the condition. Systemic forms of amyloidosis are common and may result in serious clinical consequences, while localized forms tend to be less common and clinically indolent unless they involve critical organs such as CNS. Therefore, the distinction between the two is important.

Fibril protein	Precursor protein	Associated disease
Systemic		
AL	Immunoglobulin light chain	Plasma cell disorders
AH	Immunoglobulin heavy chain	Plasma cell disorders
AA	(Apo) Serum amyloid A	Inflammation-associated amyloidosis, familial Mediterranean fever
ATTR	Transthyretin	Familial amyloidosis, senile cardiac amyloidosis
Aβ2M	β2-Microglobulin	Dialysis-associated amyloidosis
AApoAI	Apolipoprotein A I, variants	Familial systemic amyloidosis
AApoAII	Apolipoprotein A II, variants	Familial systemic amyloidosis
AApoAIV	Apolipoprotein A IV, wild type	
AApoCII	Apolipoprotein C II, variants	
AApoCIII	Apolipoprotein C III, variants	
AGel	Gelsolin, variants	Familial amyloidosis (Finnish)
ALys	Lysozyme, variants	Familial systemic amyloidosis
ALECT2	Leukocyte chemotactic factor-2	
AFib	Fibrinogen α, variants	Familial systemic amyloidosis
ACys	Cystatin C, variants	Hereditary cerebral hemorrhage with amyloidosis (Icelandic)
Abri	ABriPP, variants	Familial dementia (British)
Localized		
Adan	ADanPP, variants	Familial dementia (Danish)
Aβ	Aβ protein precursor	Alzheimer disease, Down syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch)
AαSyn	α-Synuclein	Alzheimer disease
ATau	Tau	Alzheimer disease
APrP	Prion protein	Creutzfeldt-Jakob disease, Gerstmann-Stäussler-Scheinker disease, fatal familial insomnia, kuru
ACal	(Pro)calcitonin	Medullary thyroid carcinoma
AIAPP	Islet amyloid polypeptide	Insulinoma, type 2 diabetes
AANF	Atrial natriuretic factor	Isolated atrial amyloidosis
APro	Prolactin	Pituitary amyloidoma
AIns	Insulin	
ASPC	Lung surfactant protein	
AGal7	Galectin 7	
ACor	Corneodesmosin	
AMed	Lactadherin	Aortic amyloidosis in elderly people
Aker	Kerato-epithelin	Familial corneal amyloidosis
ALac	Lactoferrin	Familial corneal amyloidosis
AOAAP	Odontogenic ameloblast-associated protein	
ASem1	Semenogelin 1	
AEnf	Enfurvitide	
ACas*	A-S2C casein	

* ACas has been identified in Animal.

Table 1.
Amyloid fibril protein classification and associated disease.

Exhaustive review of every subtype is beyond the scope of this chapter. The more common and clinically important types of amyloid protein are described below.

3.1 Amyloid light chain (AL) protein

AL type amyloid is the most common amyloid in the United States. The AL chain is a clonal immunoglobulin light chain or light chain fragment, which is produced by the uncontrolled productions of antibodies by plasma cells. Lambda (λ) type (ALλ) is more common than kappa (κ) type (ALκ) light chain. This type of amyloidosis is related to monoclonal plasma cell disorders, especially multiple myeloma or other B-lymphocyte-related disorders. If we cannot determine AL type amyloidosis to a certain disease, it is categorized as primary amyloidosis. Even if we cannot demonstrate a specific disease to AL type amyloidosis, we still see monoclonal plasma cell proliferation in these patients. In either case, we can find immunoglobulin or light chain within the serum or urine. The most commonly affected organ is the kidney. The heart, peripheral nervous system, gastrointestinal tract, and respiratory tract can also be affected. A localized form of AL type amyloid can be seen in the gastrointestinal tract [2]. Amyloid heavy chain (AH) is rarely reported but also associated with monoclonal plasma cell disorder [3].

3.2 Amyloid-associated type (AA) protein

AA type amyloidosis is the most common amyloid worldwide. AA type amyloid is an acute phase protein derived from SAP by proteolysis. AA type amyloid is made in the liver, bounds to high-density lipoprotein (HDL), and is associated with chronic inflammatory disorder. In the past, the primary cause was predominantly of infections such as tuberculosis and chronic osteomyelitis. Nowadays, with the development of antibiotics, the most common source of AA type amyloid has become noninfectious inflammatory conditions, such as rheumatoid arthritis or inflammatory bowel diseases including Crohn's disease and ulcerative colitis. Typical organs of AA type amyloid deposition are the kidney, liver, and spleen. AA type amyloidosis is also related to hereditary amyloidosis, caused by familial Mediterranean fever inherited as an autosomal recessive pattern. This is an autoinflammatory disorder characterized by frequent fever and serosal inflammation. The main affected organ is the kidney, but other organs such as the heart, spleen, and gastrointestinal tract can be affected [4].

3.3 Transthyretin amyloid (ATTR) protein

TTR is a transport protein made in the liver and choroid plexus. TTR transfers thyroxin and retinol in the blood and cerebrospinal fluid. TTR proteins can be differentiated into wild type and mutant type. TTR wild-type amyloid is associated with systemic senile amyloidosis and mostly affects heart ventricles in elderly patients. In this population, a prevalence of monoclonal gammopathy of undetermined significance (MGUS) has been reported [5]. TTR mutant type protein is related to hereditary amyloidosis. TTR mutant type protein can affect commonly peripheral nerves and other organs including the heart and gastrointestinal tract. This is inherited as an autosomal dominant pattern. The clinical course of TTR mutant type is relatively faster than TTR wild type in terms of onset and progression. Also, treatment and prognosis of two types are different. Therefore, distinguishing two types in early stage is important.

3.4 Dialysis-related amyloidosis (Aβ2M)

β2M is a light chain component of the major histocompatibility complex (MHC) class I molecules. It is found in patients on dialysis and is rarely seen in renal failure patients who have not undergone dialysis. Since β2M is catabolized in the kidney, this protein may accumulate in renal failure patients. Conventional dialysis membranes do not remove this protein, thus dialysis-related β2M deposits occur in dialysis patients. Deposits occur mostly in the carpal ligaments, synovium, and bone. Other organs such as the heart, gastrointestinal tract, liver, lungs, prostate, adrenal glands, and tongue can be affected. These days, we use polyamide high-flux membranes to remove β2M, resulting in a lower incidence of this type of amyloid.

3.5 Amyloid β (Aβ) protein

Aβ protein comes from the proteolysis of an amyloid precursor protein known as transmembrane glycoprotein and accumulates as plaque in the cerebral cortex and in the blood vessel. Deposits within the blood vessels cause CAA, which induces progressive cognitive decline and lobar hemorrhage. The most well-known disease related to Aβ is Alzheimer's disease. Rarely, familial Alzheimer's disease, which occurs in an autosomal dominant pattern, is seen. In addition, by about age 50, Down syndrome patients present with amyloid deposits in the brain similar to those of patients with Alzheimer's disease. Furthermore, a very rare form of hereditary cerebral hemorrhage with amyloidosis occurring in an autosomal dominant pattern has been reported.

Clinical symptoms are mostly nonspecific and may include headache, general weakness, edema, and weight change. Symptoms may also depend on the location and amount of amyloid deposition. Amyloid deposition in the heart causes arrhythmia, heart failure, or abnormal electric rhythm. Amyloid in the kidney eventually induces renal failure with proteinuria and uremia. If amyloid deposits within the blood vessels, it creates ischemic or hemorrhagic condition for the organ. Amyloid deposition also mimics arthritis and peripheral neuropathy. Within the brain, it causes cognitive and memory disorders seen in Alzheimer's disease or prion diseases. Localized deposition in the gastrointestinal tract has nonspecific gastrointestinal symptoms, such as dyspepsia or diarrhea. Since clinical symptoms are nonspecific, and most of the amyloid types can cause similar clinical features in the same organ; the clinical approach to amyloidosis is very limited. Therefore, a pathologic diagnosis of amyloidosis is critical.

4. Pathologic findings

Amyloid deposition is seen in the same manner within the same tissue no matter what protein it contains, except in a few cases. Grossly, amyloidosis deposition can appear as nodules and organomegaly, and sometimes it can show a pale gray to waxy color change with firm consistency. Microscopically, bright pink amorphous material deposition in extracellular space is most commonly observed under conventional hematoxylin–eosin stain. Peculiar intracellular and spheroid type amyloid depositions may be seen, but they are rare.

Since the adventitious discovery by Hans Hermann Bennhold in 1922, Congo red stain has been the gold standard of confirming the presence of amyloid protein. When properly stained, the amyloid imparts red, orange, or salmon pink color. The subsequent demonstration of apple-green birefringence confirms the diagnosis of amyloidosis. However, this two-step method still suffers low sensitivity, specificity and reproducibility and heavily depends on the interpretation of highly

experienced pathologists. For instance, aside from the inherent sampling error, negative birefringence on positive Congo red stain may result in a false-negative result, even if the stained material actually contains amyloid. This "polarization shadow" can be overcome by rotating the slide table, which may detect additional small amount of amyloid protein. The intensity of the stain is also significantly affected by the washing process in the staining protocol, resulting in low reproducibility and mandating the use of positive control tissue. In addition, Congo red also stains collagen, elastin, or even non-fibrillary materials such as eosinophils, further complicating the interpretation.

To overcome such limitations of Congo red stain, additional filters such as a fluorescein isothiocyanate (FITC) or Texas red filter can be used [6]. These filters can augment the weak signal from Congo red into red fluorescence, greatly improving the detection sensitivity. Additional fluorochrome dyes, such as thioflavin T, can also be recruited. The stain becomes highly fluorogenic only when they are bound to amyloid, which imparts a yellow-green fluorescence when it is examined under fluorescence microscopy. Because both fluorescence filters and dyes are not entirely specific for amyloid, they should be used as adjunct in the context of Congo red stain. In conclusion, the light microscopic diagnosis of amyloidosis pertains not only to the on–off signal but also to staining techniques, specimen alignment under polarized light, fluorescence microscopy, and experience of the pathologist.

Generally, histological features are similar throughout the organ, but there are still specific features for specific organs, as will be discussed below.

4.1 Heart

Heart amyloidosis is induced by various types of amyloid. AL type is the most common amyloid found in the heart, while wtATTR, which causes systemic senile amyloidosis, is the second most common amyloid in the heart. Mostly, gross features will be normal unless it is late-stage amyloidosis. Minimal to mild enlargement of the heart, along with pale and waxy changes on the external surface, can be seen. Within the heart, ventricular wall concentric thickening, including that of the septum, is seen. The epicardium, endocardium, and valves can show nodular deposits. Histologically, there is no definite pattern of amyloid deposition based on the type of amyloid, and it normally shows blight pink amorphous deposition, showing an infiltrative pattern within the interstitium. Expanding to the myocardium can cause atrophy of myocardial muscle. Also, arteriolar deposition can be seen in AL amyloidosis. Depending on the site of deposition and amount of deposition, it can cause conduction abnormality inducing arrhythmia, restrictive cardiomyopathy, and heart failure.

4.2 Kidney

The kidney is the most common organ where amyloid deposits. Various types of amyloid deposits can occur, but AA and AL type amyloids are the most common amyloid types seen in the kidney. Grossly, the kidney is firm, pale, and waxy. The size can vary between normal, enlarged, or small; if amyloid deposits within the arteries or arterioles and causes ischemia, the kidney becomes small. Histologically, there is no type-specific pattern. Amorphous bright pink deposits are mostly seen in the mesangium and capillary wall (**Figures 1** and **2**). In addition, interstitial peritubular tissue, arteries, and arterioles can be affected. Capillary wall thickening and mesangial expansion are seen. Sometimes, amyloid deposits protrude to the basement membrane of glomerular capillaries, showing discontinuity of the membrane. Expansion of amyloid within the mesangium eventually causes capillary obstruction and renal failure. Proteinuria is a very common finding among patients with kidney amyloidosis.

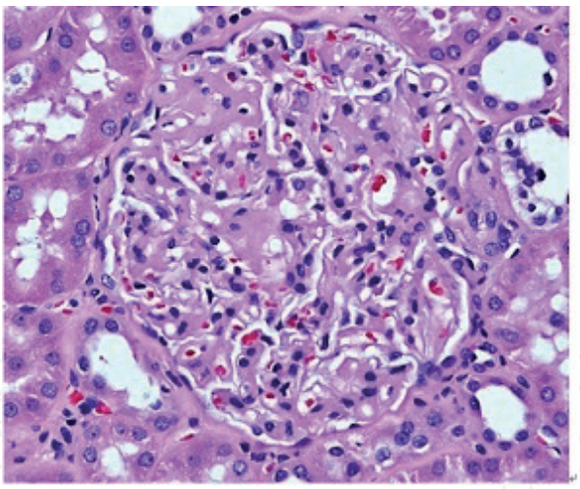


Figure 1.
Mesangium depositions of homogeneously bright pink material in kidney, H&E stain.

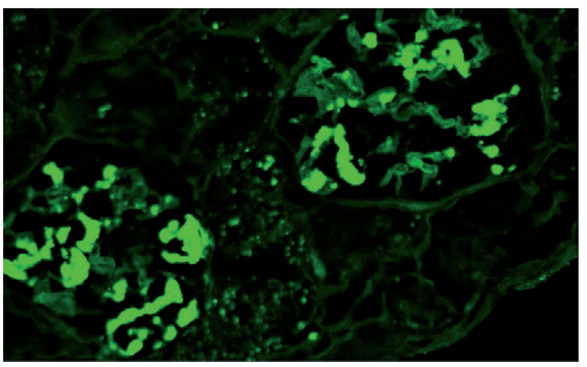


Figure 2.
Mesangium deposition of amyloid in kidney, ALλ type in immunofluorescence.

4.3 Liver

The most common amyloid type in the liver is AL and leukocyte-derived chemotaxin 2 (LECT2) type [7]. LECT 2 type amyloid (ALECT2) deposition was relatively recently found within the kidney and first reported in 2008 [8]. LECT 2 is synthesized in the liver and is a chemotaxin that attracts neutrophils. Grossly, appearance ranges from normal to moderate and massive hepatomegaly. Histologically, we can see bright pink amorphous deposition within the sinusoidal space and vessel in the portal tract. AL type tends to have a sinusoidal pattern and a vascular pattern in the portal tract, but AA type shows a vascular pattern within the portal tract [9] (**Figures 3** and **4**). A globular pattern in sinusoids has been reported in LECT 2 hepatic amyloidosis [10]. Even if there are more specific patterns depending on the type, they are not accurate, and sometimes there are overlapping patterns. Thus, confirmation with a specific stain is important. Kupffer cells and Multinucleated giant cells can be seen near the amyloid deposition. If amyloid expands, it induces hepatocyte atrophy and replaces normal hepatic tissue, causing liver failure.

4.4 Spleen

The spleen is mostly affected by AL type with plasmacytoid lymphovascular proliferation. The spleen shows two distinct gross patterns which are sago spleen and lardaceous spleen. Sago spleen has a gray, waxy, nodular appearance and is mild to moderately enlarged, and histologically, white pulp (follicles) is affected.

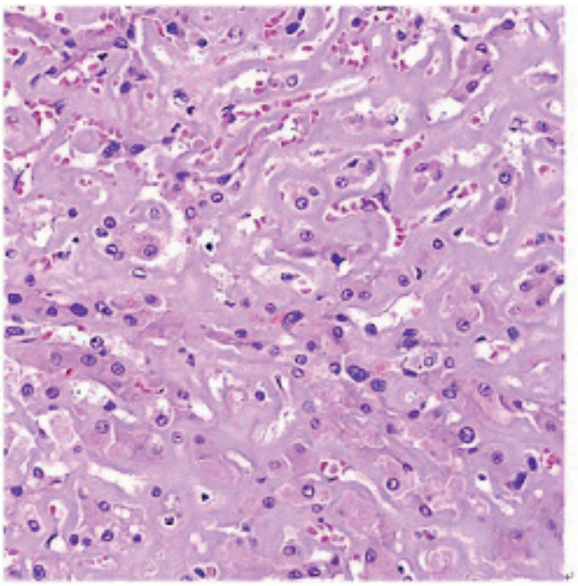


Figure 3.
Sinusoidal depositions of homogeneously bright pink material in liver, H&E stain.

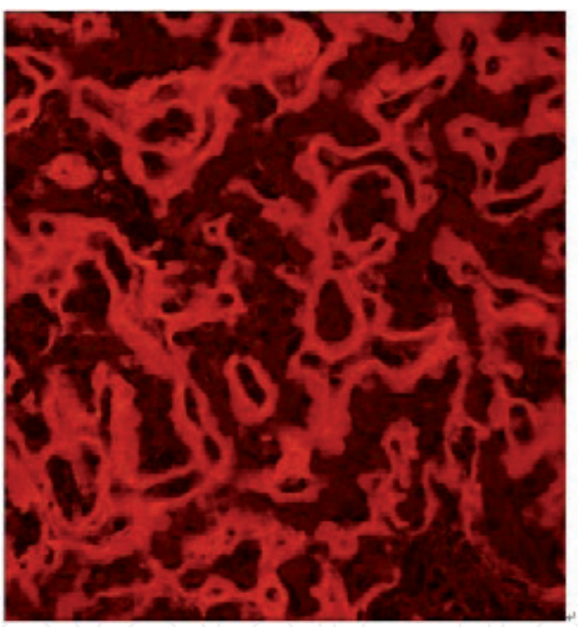


Figure 4.
Sinusoidal deposition of amyloid in liver, Congo red stain under Texas red filter.

If amyloid deposits grow, they replace the white pulp. Lardaceous spleen shows a diffuse waxy appearance and moderate to marked enlargement. Histologically, amyloid deposition is seen within the splenic sinuses and blood vessels.

4.5 Brain

The most common amyloid we see in the brain is β-amyloid. β-amyloid accumulates diffusely in the extracellular space of the cerebral cortex and is most commonly related to Alzheimer's disease. Characteristically, we can appreciate numerous extracellular depositions of amyloid plaques within the cortex (**Figure 5**). Amyloid plaques show filamentous appearance and can be demonstrated with a Congo red and a silver stain and β-amyloid IHC. Some dense amyloid plaques are surrounded by dystrophic neurites, reactive astrocytes, and microglia. Additionally, β-amyloid

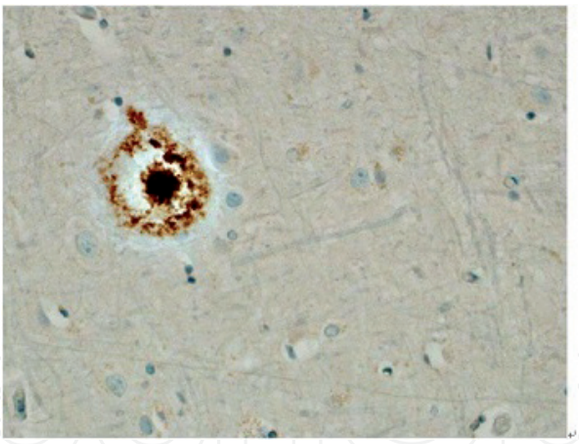


Figure 5.
Amyloid plaque seen in Alzheimer disease, β amyloid antibody in IHC.

can deposit within small-to-medium-sized arteries in the superficial cortex and leptomeningeal space and causes cerebral amyloid angiopathy. Cerebral amyloid angiopathy causes recurrent lobar hemorrhage. Furthermore, most Alzheimer's-affected brains show cerebral amyloid angiopathy as well.

4.6 Other organs

In the tongue, nodular deposition is seen, causing macroglossia. Gastrointestinal tract deposition of amyloid is common with polyps or ulcerative lesions. In the late stage, the cut surface shows yellow and waxy mural thickening. Clinically, it can cause motility disorders or stricture. Not uncommonly, vessels in the gastrointestinal tract can have amyloid deposition. In the respiratory tract, grossly nodular appearance is seen, and histologically, such depositions can be divided into four patterns including tracheobronchial, nodular parenchymal, diffuse alveolar septal, and lymphatic [11]. Skin depositions vary in size and shape, from papules to nodules and plaque. As an endocrine organ, the thyroid can present with goiter and is associated with medullary carcinoma. Localized nodular deposition in the bone causes amyloidoma. Inflammation including giant cell, lymphocyte, and spheroid structure has been reported [12]. Joint depositions are seen mimicking rheumatoid arthritis, but less synovial inflammation is seen. Bone marrow deposition is commonly seen in multiple myeloma patients. For patients on chronic dialysis, amyloid-related carpal tunnel has been seen.

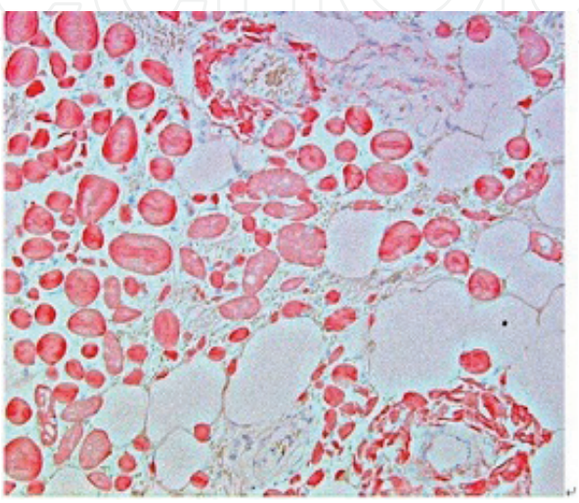


Figure 6.
Spheroid type amyloid deposition in colonic submucosa, Congo red stain.

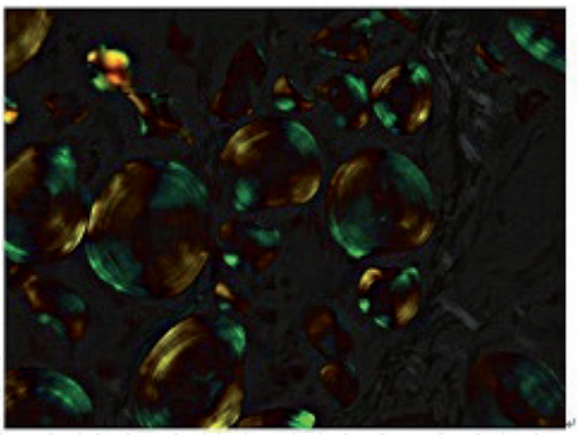


Figure 7.
Spheroid type amyloid deposition in colonic submucosa, Congo red stain under polarizer filter.

4.7 Atypical amyloid findings

Cases of intracellular amyloid deposition have been reported in few organs including cardiomyocytes, plasma cells, as well as the histiocytes and β cells of the pancreas [12–14]. Spheroid type (corpora amylacea-like) amyloid deposition is reported in pituitary adenoma, squamous cell carcinoma of the uterine cervix, and amyloidoma of the bone, jejunum, and colon [15–18]. One case of spheroid type amyloid deposition from our group in association with colon adenocarcinoma is identified [2] (**Figures 6** and 7). Current hypothesis regarding spheroid type amyloid deposition is that during the process of amyloid removal by macrophages, amyloid is packed inside the macrophage, making spheroid formations that are extracted into the surrounding tissue [18].

5. Immunohistochemistry and immunoelectron microscopy

While the Congo red stain positivity and birefringence are the gold standard of confirming amyloidosis, they do not tell what type of amyloid is deposited. Considering managements and clinical outcomes vary drastically according to the types, further studies to identify the causative protein are critical. Clinicopathologic correlation cannot substitute for amyloid typing.

Immunohistochemistry (IHC) is the most commonly utilized method for subtyping amyloidosis. IHC takes advantage of relatively specific binding properties of antibodies against different types of amyloid fibrils to illuminate the amyloid protein in tissue. A panel of antibodies for more common types can subtype the majority of amyloidosis cases. Such antibodies include those against ALλ, ALκ, AHγ, ATTR, Aβ2M, and AFib (fibrinogen). The method has been widely used due to low cost, ease of use, rapid turnaround time, and formalin-fixed paraffin-embedded (FFPE) section compatibility.

However, there is one important pitfall in IHC. Because of heterogeneity of amyloid fibrils, nonspecific staining is common, and this potentially complicates the interpretation. For instance, the antibody against Aλ is notorious for non-specific staining of amyloid other than Aλ. This diagnostic pitfall mandates the use of multiple comparative IHC stains to separate the true diagnostic positivity from the nonspecific reaction. In comparative IHC, subtyping of amyloid is determined by the specific amyloid with the strongest immunohistochemical reactivity.

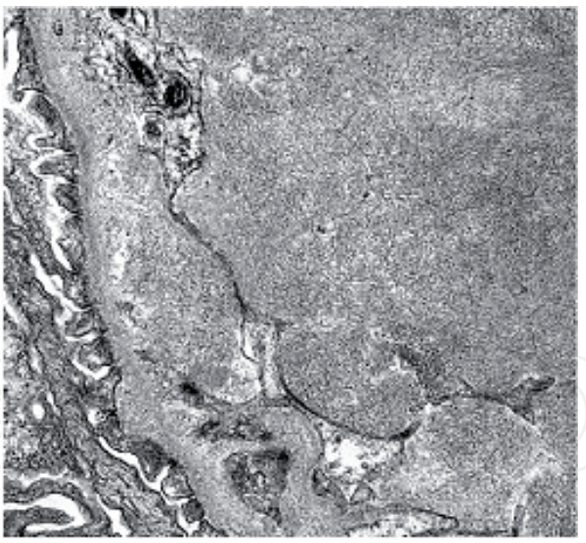


Figure 8.
Amyloid fibrils diameter of approximately 7.5–10 nm non branched fibers in kidney, EM.

Although not as commonly utilized as IHC, electron microscopy (EM) is a preferred method over Congo red birefringence or IHC in some institutions due to ambiguity of these stains in the interpretation. EM can confirm or rule out amyloidosis by visualizing amyloid fibrils in tissue as non-branching fibers with an average diameter of 7.5–10 nm. Because these fibers are considerably thicker than collagen fibers in EM, this technique can avoid diagnosing collagen fibers as amyloid fibrils, which is common in Congo red stain due to birefringence of collagen fibers in abdominal fat biopsy.

Morphologic patterns of EM have been described in amyloidosis affecting certain organs. Selective deposition in mesangial matrix and basement membrane and subepithelial "spikes" or "spicules" under podocyte foot processes are seen in glomerular amyloidosis (**Figure 8**). Amyloid deposition in tubular basement membrane, interstitial space, and vascular wall are observed in extraglomerular amyloid. However, such differences in distribution are not specific enough to indicate certain subtypes of amyloidosis.

Some authors may further utilize immunoelectron microscopy (IEM), in which immunogold stains—antibody probes conjugated with gold particles—for ALλ, ALκ, AA, and ATTR are used to subtype the amyloid fibril. These stains "decorate" the target amyloid fibers and can be seen as "beads" in the fibrillary matrix of amyloid. IEM can detect even small amounts of amyloid fibrils, at earlier stages of the disease. However, the processing deals with a very small piece of tissue and leads to a false-negative result due to the limited sampling, especially in cases where the amyloid deposits are focal. Another barrier is fixation, where architectural details are preserved by cross-linking, but at the same time loss of antigenicity may result from dehydration and embedding procedures. Therefore, alternative fixatives such as modified Karnovsky's solution rather than conventional glutaraldehyde with different protocols are used for IEM examination.

6. Molecular diagnosis

Recent advances in MS-based proteomic analysis have revolutionized detecting and subtyping of amyloidosis. The analytic method has made it possible to detect and identify new kinds of amyloid fibrils as well as previously known ones in a given specimen without direct sequencing. One such example is ALECT2. As mentioned above, LECT2 is synthesized by the liver and released into the circulation

and has uncertain physiologic roles in the cartilage and liver. It has been shown that ALECT2 is one of the major causes of kidney and liver amyloidosis after AL and AA amyloidosis, especially among Hispanics. This major amyloidosis may have been unrecognized due to a relatively indolent clinical course and limited ethnic distribution. Because serum LECT2 levels are not elevated, and no mutations are found in LECT2 gene so far, ALECT2 might have been misdiagnosed as AL or AA amyloidosis and treated as such without MS-based analysis.

The MS-based proteomic analysis utilizes techniques like laser microdissection (LMD), high-performance liquid chromatography (HPLC), and a variety of computational database tools. Although earlier HPLC- and MS-based analysis suffered from lack of specificity due to heterogeneous nature of the specimen, LMD largely overcame such diagnostic inaccuracy. LMD deals with microscopic examination of the specimen, selection of a field of interest, and microdissection of the field using laser in an attempt to achieve pure amyloid plaques. The dissected specimen can be submitted for histochemistry, IHC, or MS analysis. For FFPE specimens, an extra step for protein release similar to antigen retrieval used in IHC is applied. The released proteins are treated with a proteolytic enzyme (most commonly trypsin), and the resultant digested peptide fragments are separated by HPLC and analyzed with MS. This analytic method is based on an assumption that each human protein has their unique tryptic fragmentation patterns, which serves as a "fingerprint" of the protein. The analysis involves a previously curated database on human proteins and a number of computational algorithms to predict the amino acid sequences of the proteins that are contained in a given specimen.

Although the LMD- and MS-based proteomic analysis has demonstrated great sensitivity and specificity, they have one major pitfall. Because MS-based proteomic analysis heavily depends on human protein databases available in public, new polymorphisms or mutations may not be listed in the databases and, thus, cannot be identified using the technique. In such situations, a separate workflow to compare the newly identified mutations against previously known variants is utilized.

7. Conclusion

Amyloidosis is characterized morphologically by amorphous deposition of amyloid within tissue. The deposition is caused by aggregation of misfolded protein. Any disruptive processes in protein homeostasis (proteinostasis) can cause such misfolding and aggregation. Although different species of amyloid protein have different organotropisms and physiochemical properties, they appear remarkably similar when deposited within the target tissue. Clinical signs and symptoms of different types are largely affected by the organ where the amyloid is deposited. However, different treatment modalities and clinical courses according to the type mandate the exact subtyping of amyloid.

The confirmation and subtyping of amyloidosis heavily depend on pathologic examination of abdominal fat, minor salivary gland, or target organs. The gold standard for confirmation of amyloidosis is Congophilia and birefringence. Additional modalities such as IHC, EM, and MS can help further subclassify the type of amyloidosis.

Lately, new types of amyloidosis have been identified by MS. Atypical structure of amyloid continues to be found in various organs. Contrary to the conventional definition of amyloid, such as extracellular amorphous deposition, intracellular and spherical structure amyloids have been discovered. In addition, novel mutations of the same protein have been shown to confer totally different clinical implications.

The accumulation of new histologic findings and molecular studies will be an important key to understanding the disease mechanisms and, further on, the treatment of amyloid-associated diseases.

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