REVIEW

Amyloid in the cardiovascular system: a review

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The cardiovascular system is a common target of amyloidosis. This review presents the current clinical and diagnostic approach to amyloidosis, with the emphasis on cardiovascular involvement. It summarises recent nomenclature, classification, and pathogenesis of amyloidosis. In addition, non-invasive possibilities are discussed, together with endomyocardial biopsies in the diagnosis of cardiac amyloidosis. Finally, recent advances in treatment and prognostic implications are presented.

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Amyloidosis is a generic term that encompasses a large group of diverse diseases rather than a single disease entity. By definition, the only diagnosis of amyloidosis is morphological assessment of cytological/histological or postmortem material. Despite the fact that morphologically proteinaceous deposits are identical, these proteins are chemically different and can be identified and classified by immunohistochemical/biochemical analysis. The primary structure of these proteins is crucial to the formation of amyloid, its sites of deposition, and the clinical symptoms. Amyloid deposits may be found in any part of the body. Remarkably, amyloid may be found in the absence of clinical manifestations.

DEFINITION, NOMENCLATURE, AND CHEMICAL CHARACTERISTICS

Amyloidosis is characterised by the extracellular deposition and accumulation of insoluble fibrillar proteins, with concomitant destruction of normal tissue structure and function. Amyloid fibrils are arranged in an antiparallel conformation with a β pleated sheet structure.[1–3] It is recommended that amyloid and amyloidosis should be classified by the fibrillar protein forming the amyloid deposits. The current nomenclature of amyloidosis is based on the nature of the major fibrillar protein, which is designated protein A, followed by an abbreviation of the protein name. Eighteen proteins, 19 if lactoferrin is included, have been identified to date.[4–6] Table 1 summarises the main protein types causing amyloidosis.

> "It is recommended that amyloid and amyloidosis should be classified by the fibrillar protein forming the amyloid deposits"

In addition to the fibrils, which characterise the material as amyloid, it contains other components, which are common to most types of amyloid. These include serum amyloid P component, amyloid enhancing factor, and glycosaminoglycans. Other basement membrane components, including fibronectin, laminin, and collagen IV, may also be found in amyloid deposits.[7–9]

PATHOPHYSIOLOGY

Although at least 18 different amyloidogenic proteins have been recognised, identical processes are probably involved in the formation of the amyloid fibrils, which are remarkably similar to each other, despite the biochemical differences. Protein species with a conformation that allows ordered self assembly are needed for fibril formation. The process is performed either by partial unfolding of the native protein (for example, light chain immunoglobulin), by proteolytic cleavage (for example, lactadherin), or by adoption of a secondary structure in a peptide with a random coil. Many precursors of amyloid play a role as molecular carriers, others form multipeptide complexes.[3 10 11]

The mechanism by which amyloid aggregation causes tissue damage and consequent organ dysfunction has been widely discussed and studied. Amyloid deposition can disturb the tissue architecture and lead to organ dysfunction. Local cytotoxicity and interactions with local receptors can also influence organ function.[3 10] Free radical injury mechanisms have been suggested in amyloidogenesis in several types of amyloidosis.[12] Recently, it has been shown that light chain proteins from patients with amyloid cardiomyopathy can alter the cellular redox state in cultured cardiomyocytes.[13] In addition, both in vivo and in vitro evidence has shown that amyloid can induce apoptosis.[14 15]

CLINICAL CLASSIFICATION

In clinical practice, amyloidosis is classified as primary, secondary, hereditary, and age related.[16] Primary (idiopathic, systemic) amyloidosis appears with no antecedent or coexisting disease, it involves mesenchymal organs such as the cardiovascular system, gastrointestinal tract, and muscle tissue, and tends to form nodular deposits. Cardiac involvement is common.[16–18] Secondary (reactive) amyloidosis is associated with chronic diseases, and has a tendency to deposit in parenchymal organs such as the liver, spleen, and kidneys. The heart is involved only rarely.[16 19] The hereditary amyloidoses are usually inherited in an autosomal dominant fashion,[20 21] with only a few autosomal recessive forms such

Abbreviations: ANF, atrial natriuretic factor; IAA, isolated atrial amyloidosis; SAA, serum amyloid A; SSA, systemic senile amyloidosis; TTR, transthyretin

Table 1 Main protein types causing amyloidosis with the emphasis on cardiovascular system involvement

Amyloid protein	Precursor	Distribution	Syndrome
AL	**Immunoglobulin light chain**	Systemic/localised	Primary/myeloma associated
AH	**Immunoglobulin heavy chain**	Systemic/localised	Primary/myeloma associated
AA	**Serum amyloid A**	Systemic	Secondary
Aβ_2 Microglobulin	**β_2 Microglobulin**	Systemic	Secondary
ATTR	**Transthyretin**	Systemic	Senile systemic/familial
AANF	**Atrial natriuretic factor**	Localised	Atrial isolated
AApoA-I	**Apolipoprotein A-I**	Localised/systemic	Aortic/familial
AApoA-II	Apolipoprotein A-II	Systemic	Familial
Amed	**Lactadherin**	Localised	Aortic
Agel	Gelsolin	Systemic	Familial
Alys	Lysozyme	Systemic	Familial
Afib	Fibrinogen α chain	Systemic	Familial
Acys	Cystatin C	Systemic	Familial
Aβ	Aβ Protein precursor	Localised	Alzheimer's disease, aging
AprP	Prion protein	Localised	Spongiform encephalopathies
Abri	ABri protein precursor	Localised	Familial dementia
Acal	(Pro)calcitonin	Localised	Thyroid tumours derived from C cells
AIAPP	Islet amyloid polypeptide	Localised	Langerhans islets, insulinomas
Apro	Prolactin	Localised	Prolactinomas, pituitary in elderly
Ains	Insulin	Localised	Iatrogenic
Aker	Kerato-epithelin	Localised	Familial, cornea
Alac	Lactoferrin	Localised	Familial, cornea

Proteins involved in the cardiovascular system are in bold.

as familial Mediterranean fever[22] and familial corneal amyloidosis.[23] Cardiac involvement is rare and usually occurs late in the disease.[16 21 24] Age related (elderly) amyloidosis is classified as either the isolated atrial form or the systemic senile type. Age is the only risk factor. The incidence increases with aging and is not related to concurrent disease. The heart is the main target.[25–29]

CARDIAC CLINICAL MANIFESTATIONS

In general, the symptoms of amyloidosis are non-specific. Cardiac amyloid involvement may simulate cardiomyopathy, congestive heart failure, coronary heart disease, valvular heart disease, or arrhythmia.[16–18 30–34] Table 2 lists the clinical features of cardiac amyloid involvement. Unusual features include spontaneous resolution of hypertension and hemi blocks.[34]

Initial symptoms that might lead to the suspicion of cardiac amyloidosis include fatigue, dyspnoea, purpura, macroglossia, atypical chest pain, hepatomegaly, peripheral oedema, arrhythmia, and systolic murmur. Predominantly right sided heart failure is the most common clinical feature. In addition, coronary heart disease is often incorrectly diagnosed.[16 34 35]

Of note is the male and age preponderance: 60–65% of patients with amyloidosis are men and only 1% of patients are younger than 40 years of age.[35]

Table 2 Cardiac clinical manifestations of amyloidosis

Clinical characteristics of cardiac amyloid involvement
Dilated cardiomyopathy (cardiomegaly, predominant systolic dysfunction)
Restrictive cardiomyopathy (slight cardiomegaly, predominant diastolic dysfunction, stiff heart syndrome)
Congestive heart failure
Electrocardiographic disorders (rhythm abnormalities, low voltage QRS complex, sick sinus syndrome, pseudoinfarct pattern, atrioventricular and ventricular conduction abnormalities)
Coronary insufficiency (myocardial infarction, angina pectoris)
Valvular dysfunction
Pericardial tamponade
Enhanced sensitivity to digitalis glycosides
Atrial thrombosis—embolisation

DIAGNOSIS

The expanding number of amyloidogenic proteins causes difficulties in formulating a correct diagnosis. The identification of amyloidogenic proteins has paramount importance for treatment and prognosis. The diagnostic approach should be multidisciplinary and include clinical examination, biochemical tests, imaging, and genetic analysis to assess the extent of the disease and its complications.[36 37]

Misdiagnosis

Unfortunately, because many of the clinical signs of amyloidosis are non-specific, they are often misinterpreted. Fatigue may be misdiagnosed as functional or stress related. Purpura may be misdiagnosed as senile purpura or purpura simplex. Involvement of submandibular salivary glands may be interpreted as submandibular lymphadenopathy and consequent xerostomia as Sjögren syndrome. Increased sedimentation often leads to a diagnosis of polymyalgia rheumatica.[35]

Non-invasive tests

A diagnostic approach to patients with suspected cardiac amyloidosis might include the following non-invasive methods to help in establishing the diagnosis: electrocardiography, echocardiography including Doppler mode, chest radiography, magnetic resonance imaging, radionuclide imaging of radiolabelled amyloid P protein, protein electrophoresis to search for monoclonal immunoglobulins in serum and urine, abnormal transthyretin or amyloid A protein in serum, and genetic testing for suspected hereditary amyloid disorders.[16 18–20 34 38 39]

''The diagnostic approach should be multidisciplinary and include clinical examination, biochemical tests, imaging, and genetic analysis to assess the extent of the disease and its complications''

The first line non-invasive test for both the diagnosis and assessment of cardiac involvement is echocardiography with Doppler mode.[35 38] In the case of suspected primary amyloidosis, immunoanalysis of the serum and urine to detect monoclonal immunoglobulin light chains is the non-invasive diagnostic test of choice.[18 35]

The serum hepatocyte growth factor concentration has been reported to be significantly higher in patients with both primary and secondary amyloidosis,[40] but further multicentric studies are needed. Recently, the diagnostic potential of (trans,trans)-1-bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)-styrylbenzene has been considered. This Congo red non-toxic derivative can bind to amyloid both in vivo and in vitro and it can be evaluated in scintigraphic studies.[41]

Morphological diagnosis of amyloidosis in general

Amyloid can be diagnosed and classified in any affected tissue specimen by means of special stains and biochemical analysis. The morphological diagnosis is of paramount importance and cannot be replaced by clinical, biochemical, or radiological procedures. Fat aspiration biopsy introduced by Westermark and Stenkvist in 1973 is the most common tool to diagnose systemic amyloidosis.[42] Fat aspiration biopsy is simple, easy to perform and repeat, and bears a negligible risk of complications. However, fat aspiration biopsy is less sensitive than kidney and rectal biopsy. Moreover, the sample should not contain only fat droplets. Alternatively, if sufficient material is present, it can be used for further immunohistochemical, electron microscopical, and molecular analyses.[43–47] Another standard procedure for the confirmation of the diagnosis of systemic amyloidosis is rectal biopsy,[48–50] and amyloid is commonly diagnosed by renal biopsy.[51 52] Furthermore, sural nerve, skeletal muscle, and gastrointestinal biopsy samples other than rectal can also be used in the diagnosis of amyloidosis.[44 49 53 54] Table 3 provides a diagnostic algorithm for the diagnosis and classification of amyloidosis in biopsy samples.

Biochemical analysis from biopsy material

Both formalin fixed and fresh/frozen tissue samples can be used for the further biochemical analysis of amyloid. Reversed phase high performance liquid chromatography is the method of choice for the purification and analysis of amyloid proteins extracted from formalin fixed tissues. In contrast sodium dodecyl sulfate polyacrylamide gel electrophoresis based western blotting appears to be the better method for fresh tissue analysis.[55] Furthermore, the determination of chemical composition through amino acid sequencing or mass spectroscopy from formalin fixed specimens is available.[56]

Diagnosis of cardiac amyloidosis

Unfortunately, because of the diversity of symptoms, cardiac amyloidosis is diagnosed predominantly after death,[57] although the tentative diagnosis of cardiac amyloidosis can easily be confirmed by myocardial biopsy.

Endomyocardial biopsies

Endomyocardial biopsy was found to be a safe and effective method for assessing cardiac amyloid involvement.[58–61] According to American College of Cardiology/American Heart Association criteria, endomyocardial biopsy is recommended in patients with heart failure in whom an inflammatory or infiltrative disorder of the heart is suspected.[62] Fat aspiration biopsy is a possible alternative to endomyocardial biopsy in cases of primary amyloidosis.[36] Another alternative is rectal biopsy, although this approach has a lower positivity, ranging from 58% to 80%, and thus cannot substitute completely for endomyocardial biopsy.[57 59 63] In cases with known amyloidosis, as demonstrated by a positive biopsy from extracardiac tissue with echocardiography and other signs indicating cardiac involvement, endomyocardial biopsy is not essential.[57 59] However, endomyocardial biopsy remains the method of choice to diagnose cardiac amyloidosis when non-invasive tests give equivocal results.[59 61 64]

In addition, immunohistochemical analysis to characterise amyloid protein is feasible. In most cases, the tissue obtained is sufficient for such an analysis.[58] Endomyocardial biopsy also enables the degree of myocardial damage and cellular changes to be assessed.[59 61]

> ''Endomyocardial biopsy is recommended in patients with heart failure in whom an inflammatory or infiltrative disorder of the heart is suspected''

In a series of 454 endomyocardial biopsies, systemic amyloid was found in 7%. The yield of endomyocardial biopsies is increased if at least four biopsy specimens are obtained. Importantly, cardiac involvement is interstitial, plentiful, and easily recognised compared with the almost exclusively vascular, mild, and focal pattern seen extracardially.[59] Unfortunately, small amyloid deposits can be missed at light microscopy using Congo red staining.[61 65] Congo red fluorescence is recommended in all Congo red negative suspicious tissue samples.[66] In such cases, electron microscopy is of paramount importance and positive results have been reported in Congo red negative cases.[61]

Table 3 Diagnostic algorithm for biopsy samples to diagnose and classify amyloid

(1) Haematoxylin and eosin stain, Congo red polarisation
(2) Congo red fluorescence
If negative:
(3A) Serial sections of the whole sample (3B) Electron microscopy
(3AA) Haematoxylin and eosin stain, Congo red polarisation
(3AB) Congo red fluorescence
If positive:
(4) Immunohistochemical classification

Morphology

Macroscopy

Amyloid is either indistinct or associated with a waxy cut surface. Localised forms can lead to organ enlargement and can imitate tumours.

At necropsy, the reaction with Gram's iodine followed by treatment with 10% sulfuric acid will show amyloid deposits as a blue/violet colour. Either fresh tissue or frozen sections can be used to demonstrate amyloid deposits.[67]

Heart macroscopy

Hearts infiltrated by amyloid are usually only moderately enlarged, weighing 400–850 g, although giant hearts of over 1000 g have also been described.[30 33 68] Interestingly, the mean cardiac weight in senile involvement was significantly higher than that seen in subjects with primary (even fatal) involvement.[33] Most infiltrated hearts are firm, rubbery, and non-compliant, mimicking hypertrophic cardiomyopathy—that is, the walls of heart chambers are thickened, but the ventricular lumina are not dilated. About one third of hearts have dilated chambers imitating congestive cardiomyopathy. Importantly, about 15% of hearts show no macroscopically visible changes.[69 70] In the senile form, atrial deposits are grossly discernible as bead-like, semitranslucent nodules (fig 1A).[30 70]

Light microscopy and special stains

General pattern and stains

At light microscopy, all forms of amyloid deposits are amorphous and homogenous, with pale eosinophilic areas, when stained with haematoxylin and eosin (fig 1B, C).

Since the 19th century, amyloid deposits have been known to stain metachromatically if aniline dyes such as methyl or

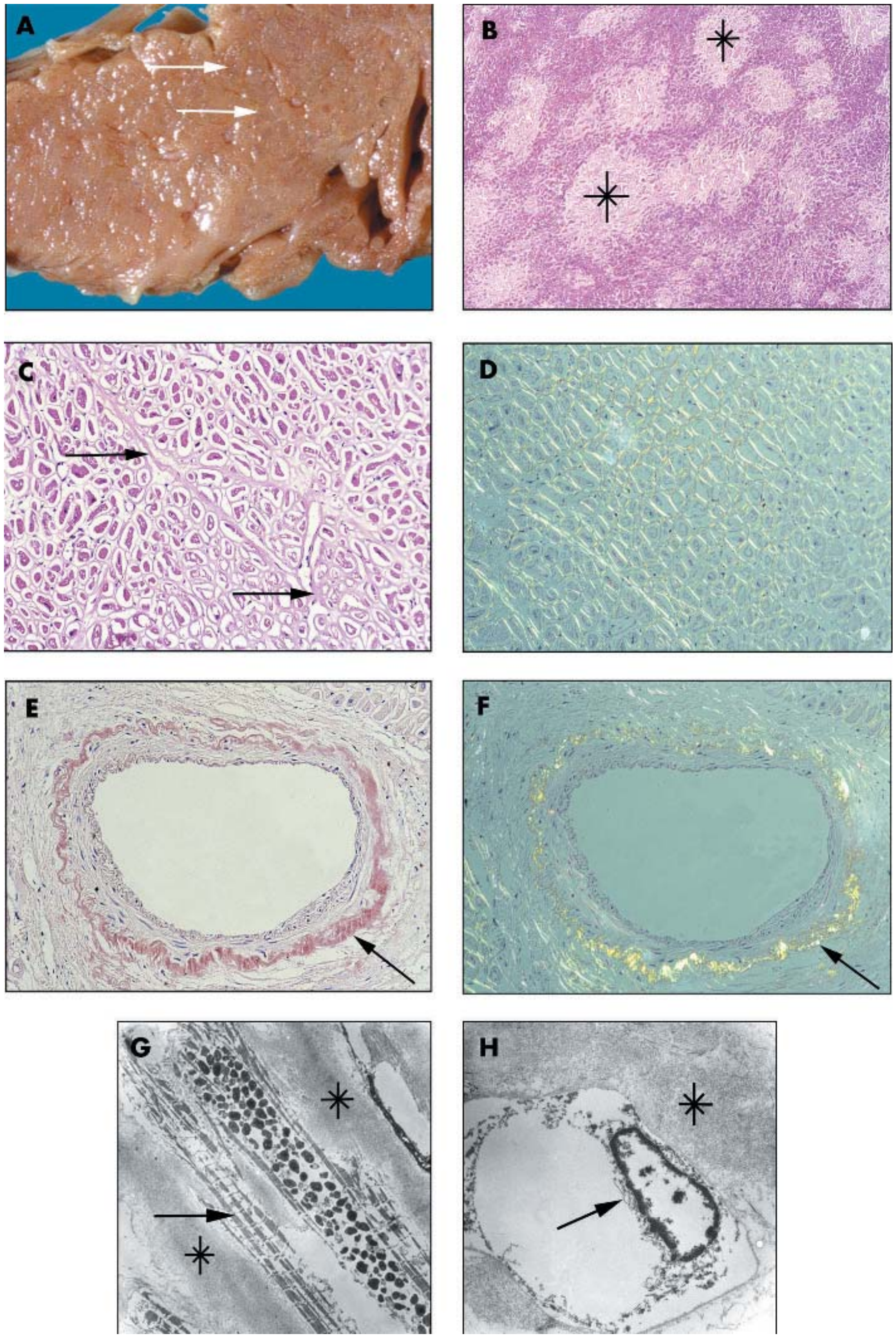

Figure 1 Example of a heart with amyloidosis. (A) Macroscopic view showing nodules with amyloid (arrows). (B) Microscopic view showing eosinophilic areas containing amyloid (asterisks); original magnification, ×100. (C) Microscopic view showing eosinophilic bands, containing amyloid, around individual cardiomyocytes (arrows); original magnification, ×100. (D) Microscopic view (same figure as C) showing apple green birefringance under polarised light; original magnification, ×100. (E) An intramyocardial artery showing Congo red stained amyloid (arrow); original magnification, ×400. (F) An intramyocardial artery (same figure as E) showing apple green birefringance under polarised light; original magnification, ×400. (G) Amyloidosis is characterised by the deposition of fine fibrillar material (asterisk) in and around cardiomyocytes (arrow); original magnification, ×10 000. (H) Amyloidosis is characterised by the deposition of fine fibrillar material (asterisk) around small vessels; arrow, endothelial cell; original magnification, ×15 000.

crystal violet are used. At present, Congo red is the standard staining method. Congo red stained amyloid has an orange or red colour on light microscopy and has apple green birefringence under polarised light (fig 1D–F). However, Congo red is not specific, because it also stains eosinophil granules, enterochromaffin granules, Paneth cell granules, elastic fibres, collagen, and foreign materials such as chitin, fungal constituents, and plant components. Certain pitfalls can be avoided by alkaline Congo red modification.[66 71–76] Most of the other stains available are not recommended to be used alone and are listed with decreasing specificity and sensitivity: Sirius red, thioflavine T, toluidine blue, *p*-dimethylaminobenzaldehyde-nitrite, alcian blue, and crystal violet.[67 74 75] Alternatively, a panel of special stains has been recommended to increase the sensitivity in certain cases because of possible staining variability.[76] Importantly, prolonged formalin fixation of amyloid tissue may abolish or lessen its staining reactions. Some authors stated Sirius red as superior in cardiac amyloidosis, particularly for identifying very small deposits in the isolated atrial form.[29]

Cardiac involvement pattern

The degree of involvement of different parts of the heart—endocardium, myocardium, pericardium, valves, coronary arteries, and veins—is not uniform in the different forms of amyloidosis.[30 58 59] However, amyloid patterns are of limited value in the diagnosis in individual patients.[58] Perifibrous/pericellular is the most common pattern seen, and nodular and mixed patterns have also been described.[17 58 59 70] Interestingly, atrophy of the surrounding myocytes and fibrosis of the conduction system have been noted in relation to amyloid deposition.[77 78] The myocytes often reveal perinuclear vacuolation,[57] although this is a non-specific feature also found in hypertrophy related to increased glycogen storage. Amyloid deposits in the vessel wall are predominantly segmentally distributed and cause luminal narrowing.[57]

Immunohistochemistry

The immunohistochemical classification of amyloid deposits is a useful tool with increasing importance in amyloid diagnosis, and a large number of anti-amyloid fibril protein antibodies are commercially available. However, the increasing number of amyloid fibrillar proteins identified may be demanding in routine practice. The following spectrum of fibrillar proteins is recommended to test for the most common systemic amyloidosis: amyloid A, amyloid of apolipoprotein A-1, amyloid of fibrinogen α chain, amyloid of light chains, amyloid of lysozyme, amyloid of transthyretin, and amyloid of β2 microglobulin origin. Table 4 lists a differential diagnostic panel of antibodies to be used in heart and vessel amyloid assessment.[47 79–83] Recently, an immunogold technique that enables typing of amyloid in difficult clinical cases was reported.[84]

> "The immunohistochemical classification of amyloid deposits is a useful tool with increasing importance in amyloid diagnosis, and a large number of anti-amyloid fibril protein antibodies are commercially available"

In routine histopathology practice we face both false negativity and positivity of the specimens. The main reason for false positivity is when amyloid fibril proteins originate from serum proteins, which are then detected in the tissues although they are not related to amyloid. In contrast, the cleavage products of amyloid fibrillar proteins lose their epitopes or differ greatly from the precursor protein structure. Immunoelectron microscopy is an additional useful tool that can easily distinguish contaminating labelled protein structures.[47 80 82]

Table 4 Commercially available antibodies against amyloid protein fibrils in general/cardiovascular amyloidosis diagnostics

Antibody	Clonality/clone	Distributor
κ Light chain	Polyclonal	Dako, Glostrup, Denmark
λ Light chain	Polyclonal	Dako
AA Amyloid	Monoclonal/mc1	Dako
β2 Microglobulin	Polyclonal	Dako
Atrial natriuretic peptide	Polyclonal	Biogenesis, Poole, Dorset, UK
Transthyretin	Polyclonal	Dako

Electron microscopy

General ultrastructure

Ultrastructurally, amyloid consists of a loose meshwork of 7–10 nm rigid, non-branching, hollow fibrils of indeterminate length. The fibrils measure from 30 to more than 1000 nm in length. These fibrils are usually found in extracellular spaces and aggregate in a crossed β pleated sheet conformation.[1 2 85 86]

Cardiac ultrastructure

In the myocardium, pericellular encasement non-branching fibrils are found. Fibrils are adjacent to the basement membrane of myocytes, some of which can be completely surrounded by fibrillous material (fig 1G, H). The amyloid fibrils are in close proximity to the basement membrane of the myocytes. Furthermore, the deposition of amyloid is associated with a focal increase of mitochondria.[57 87] Electron microscopy can detect amyloid when histochemical stains such as Congo red are still negative.

CARDIOVASCULAR AMYLOIDOSIS AS A PART OF PRIMARY AMYLOIDOSIS

In primary amyloidosis, clonal plasma cells secrete monoclonal immunoglobulin light chains, which are deposited predominantly in the kidney, heart, and nerves.[11 18] Symptomatic cardiac involvement is present in a quarter to half of patients with primary amyloidosis and is the major prognostic factor.[18 34] Importantly, a cardiac cause of death is the most common amyloid related death in primary amyloidosis, and is seen in 40% of patients in the form either of congestive heart failure or arrhythmia.[33 64 88 89] Congestive heart failure is the most frequent cardiovascular manifestation. Conduction system abnormalities and heart blocks are the second most frequent complications.[33 34 88] Stenoses and obstructions in the intramural coronary arteries by amyloid deposits can lead to ischaemic disease, which differs in prognosis. Interestingly, epicardial coronary artery involvement and interstitial myocardial involvement were absent or mild in these cases.[90–92] In addition, non-sclerotic valves showed amyloid deposits in primary amyloidosis.[93] Pericardial effusion is a common symptom. However, constrictive pericarditis and cardiac tamponade resulting from amyloid deposits are unique.[94 95] Orthostatic hypotension caused by amyloid deposits in nerves and ganglia was also revealed.[88] Uniquely, cardiac denervation was described in primary amyloidosis, with no other cardiac symptoms.[96] In general, cardiac involvement in the absence of other organ deposits is rare.[34]

Systemic vascular involvement often leads to obstruction and consequent ischaemia.[17 97] In fact, vascular involvement is very common (around 90%), affecting medium to large arterioles and small arteries. Amyloid is then deposited in the media and adventitia, thereby thickening the wall. Interstitial fibrosis and degeneration of myocytes as a result of ischaemia was seen in the vicinity of these vessels.[20 33 58 77 83 98]

SECONDARY AMYLOIDOSIS

With the decrease of chronic infectious diseases, such as tuberculosis and osteomyelitis in the developed world, secondary amyloidosis has become rare and is found mainly in association with diseases such as rheumatoid arthritis and inflammatory bowel disease.[16] A tumour related form is also diagnosed and recently haemodialysis related amyloidosis was described.[83 99] Amyloid is formed from serum amyloid A (SAA), an acute phase protein produced in response to inflammation. Several forms of SAA have been identified in human plasma. SAA1 predominates in the formation of amyloid A deposits.[100 101] The major constituent protein in haemodialysis related amyloidosis is β_2 microglobulin.[99]

Cardiac involvement may occur; however, significant deposition of amyloid in the heart is unusual and is rarely the cause of death.[17 19 102] Although myocardial infiltration is unusual, there have been case reports of extensive cardiac involvement, even with cardiac failure and arrhythmia, in secondary amyloidosis in such diseases as juvenile rheumatoid arthritis, sarcoid, psoriatic spondyloarthropathy, and multifocal neurone neuropathy.[103–106] Vascular involvement is less frequent, mostly involving intramyocardial small vessels. In contrast, in β_2 microglobulin amyloidosis, only vascular and endocardial involvement was described.[20 83 98 104] Involvement of the conduction system was described accompanying juvenile rheumatoid arthritis and psoriatic spondyloarthropathy.[104 105] Amyloid deposits were also found in post rheumatic sclerotic valves.[107 108]

AGE RELATED AMYLOIDOSIS

Age related amyloidosis is either isolated atrial or systemic senile amyloidosis (formerly designated senile cardiac amyloidosis). In addition, transthyretin isoleucine 122 amyloidosis is sometimes thought to be age related, despite it being an autosomal recessive disease.[64]

Isolated atrial amyloidosis

Isolated atrial amyloidosis (IAA) is a common postmortem finding in the elderly and has been reported to occur in as many as 90% of those $\geqslant$ 90 years of age.[26 28 29] IAA first appears in the third decade and its prevalence increases linearly by 15–20% with each subsequent decade.[28 29 109] Interestingly, the prevalence of IAA was found to be significantly higher in the chronic rheumatic heart and in mitral valve disease.[81 109] Women have a higher prevalence of IAA.[29 81]

Atrial natriuretic peptide (ANF) is the major protein subunit of the amyloid fibril in IAA.[110] IAA can theoretically be found at any site expressing ANF; however, only atria are targeted in IAA.

> ''Isolated atrial amyloidosis is a common postmortem finding in the elderly and has been reported to occur in as many as 90% of those $\geqslant$ 90 years of age''

Although the clinical relevance of IAA is not clear, patients with IAA are more likely to develop atrial fibrillation.[26 81]

Left atrial deposits are more pronounced. In addition, deposits are usually found in the auricles, predominantly beneath the endocardium. Microscopically, very thin deposits occur along the sarcolemma of atrial myocytes, with a tendency to coat them. Small intracellular deposits are frequently present in these cells. Associated myocyte hypertrophy has been described. Of note, in Rocken's study, IAA amyloid deposits correlated inversely with the degree of interstitial fibrosis.[81] Very small vessels are often involved.[25 27 29 109] Amyloid deposits were found to be 100% positive for ANP and four of 40 cases showed transthyretin immunopositivity.[81] However; there is no correlation between the degree of IAA and the presence of systemic senile amyloidosis (SSA).[29]

Systemic senile amyloidosis

The prevalence of SSA increases with each decade above the age of 60, occurring in 25% of people over the age of 80 years, and thus is the most common form of systemic amyloidosis.[25–28] However, symptomatic senile cardiac amyloidosis was reported in a patient as young as 57 years.[89] The major constituent of the amyloid fibrils in SSA is derived from normal transthyretin (TTR), previously known as prealbumin.[28] TTR is also the major precursor for several heredofamilial forms of amyloidosis and, to date, more than 40 point mutations have been found.[27 28] However, TTR mutation is not required for the deposition of senile systemic amyloid and TTR in SSA has a normal primary structure.[28]

Although initially cardiac involvement was thought to be an incidental finding in SSA, its extent is similar to that found in primary amyloidosis, although it does not carry the same grave prognosis.[25 58 70 111] Only a small proportion of patients develop cardiomegaly with heart failure and/or arrhythmia.[27]

The extracardiac involvement is limited to the aorta, pulmonary vessels, and pulmonary alveolar septa.[25 27] In the heart, both atria and ventricles are involved, and the small to medium sized vessels are involved to a lesser extent (4–27%). Microscopically, large diffuse or multifocal, predominantly nodular, deposits are present between muscle bundles. Deposits become less homogeneous in density and more fibrillar as the deposits increase in size. Adjacent cells are often atrophied. In contrast to primary amyloidosis, the conduction system is not affected.[20 25 58 70 112]

HEREDITARY AMYLOIDOSIS

Familial amyloidosis occurs throughout the world and in all ethnic groups. It encompasses an extremely broad spectrum of clinical manifestations but is dominated by peripheral neuropathy, renal failure, intracranial haemorrhage as a result of cerebral amyloid angiopathy, and ocular deposition.[21 24] Cardiopathic forms are characterised by severe heart failure and arrhythmias.[21 65 113–116] Symptomatic valvular amyloid involvement has also been reported.[117]

The most common form is caused by mutant TTR. Although neurological symptoms are predominant, several variants with cardiac involvement have been described.[21 65 114–116 118 119] In addition, a form caused by a point mutation in codon 122 of the TTR gene, which results in substitution of isoleucine for valine during TTR translation, was found in 3.9% of the Afro-American population, with a 7.5 fold greater risk of developing cardiac amyloidosis in heterozygous subjects.[120] Interestingly, patients with familial amyloid polyneuropathy in Japan revealed wild-type TTR in their hearts.[121]

VALVULAR AMYLOID INVOLVEMENT

Microscopic amyloid deposits were described in 15.5–88% of sclerotic valves with simultaneous occurrence of hyalinisation and calcification.[103 107 108 122] Biochemical and immunohistochemical assessment failed to identify all known amyloidosis related proteins in valvular deposits.[107 123] Interestingly, an association between valvular amyloid deposits and old thrombotic material was proposed.[124] Furthermore, amyloid was found in porcine bioprosthetic cardiac valves after explantation following longterm implantation.[125]

AMYLOID INVOLVEMENT OF VESSELS

Senile aortic amyloid

Occurring in nearly all people over 50 years of age, aortic amyloid appears to be the most common form of localised amyloid. Biochemically, aortic amyloid is distinct either from ANP present in IAA or from TTR in SSA.[126 127] Recently, the lactadherin derived protein, medin, was purified from medial amyloid deposits in aortas and temporal arteries,[128 129] whereas intimal amyloid is derived from apolipoprotein A-I.[130 131] Serum amyloid A isotypes were detected in atherosclerotic lesions.[132]

> ''Occurring in nearly all people over 50 years of age, aortic amyloid appears to be the most common form of localised amyloid''

Three different forms involving the media, intima, and adventitia have been described so far. The most common form is in the media, followed by intimal and adventitial deposition. The clinical relevance is not clear yet. No casual relation between aortic amyloid and hypertension or dissecting aneurysm was observed. The thoracic aorta is predominantly involved in the medial form: nodules and thin streaks in the inner half of the media are mostly in the proximity of elastin fibres. Intimal amyloid is associated with atheromatous lesions and appears as irregular lumps. Adventitial amyloid is either in the connective tissue or in the walls of the vasa vasorum.[27 126 129]

Other forms of vascular amyloidosis

Senile amyloid angiopathy has been described in a variety of vessels. Its biochemical characteristics remain unknown, but similarities between aortic medial amyloid and medial amyloid of the common carotid and temporal arteries were reported.[27 129 131] Senile vascular amyloidosis derived from the N-terminus of apolipoprotein A-I reported in dogs has yet to be documented in humans.[27 133] Interestingly, amyloid involvement was greater in the veins than in the arteries in senescence accelerated mice.[134]

TREATMENT

The therapeutic approach depends on the type of amyloidosis and the stage of the disease, so the precise diagnosis is of principal importance. The treatment of amyloidosis is specifically directed at the amyloidogenic process, and supportive treatments directed to consequent organ dysfunctions.[18]

Supportive treatment of cardiac failure includes diuretics, whereas calcium channel blockers, β blockers, and digoxin are contraindicated in cardiac amyloidosis.[16] Patients with arrhythmia may benefit from a pacemaker.[16]

In primary amyloidosis, chemotherapy regimens of melphalan and prednisone have been used for decades and are the most successful. Alternative drugs under consideration and in trials are 4′-iodo-4′-deoxydoxorubicin, vincristine, dexamethasone, and α interferon.[16 18 135] Cardiac transplantation with supportive chemotherapy is under consideration,[16 136] and stem cell transplantation is another treatment option.[135 137]

Secondary amyloidosis requires aggressive treatment of the underlying inflammatory or neoplastic process.

Currently, no definitive treatment is available in nonhereditary, age related amyloidosis; however, patients may benefit from supportive treatment, including a pacemaker.[16 64]

To date, no effective treatment has been reported for the most common hereditary amyloidosis, TTR amyloidosis, apart from liver transplantation. However, the onset of cardiomyopathy can be prevented if liver transplantation is carried out before cardiac involvement.[65] Successful combined heart–liver and liver–kidney transplantation was reported for hereditary TTR and apolipoprotein A-I amyloidosis, respectively.[138 139]

Future perspectives include treatments based on precursor stabilisation (transthyretin), prevention of formation by crosslinking, elimination of the synthesising cells (light chains), and immunisation to induce host mediated reaction (light chains).[37 140 141]

PROGNOSIS

The prognosis varies according to the type of amyloidosis, the stage of the disease, and the age of the patient at the time of diagnosis. Primary amyloidosis has the worst prognosis, which is exacerbated by multisystem involvement and cardiac involvement in particular.[142] Histologically, the worst prognosis is connected with the presence of nodular deposits, thick perimyocytic layers of amyloid, and small myocyte diameters in endomyocardial biopsy.[61] The overall median survival after diagnosis is less than two years in most. In secondary amyloidosis, the underlying chronic disease affects the prognosis, and hereditary amyloidoses vary in prognosis according to the specific mutation.[16 18 34] In addition, heart rate variability assessed by Holter monitoring was found to be a possible predictor of mortality in patients with both primary and secondary amyloidosis involving the heart.[143] Echocardiography should be a routine part of the assessment of cardiac involvement and predicting prognosis.[35]

Take home messages

- The cardiovascular system is often affected by amyloidosis—the extracellular deposition and accumulation of insoluble fibrillar proteins
- The heart is targeted most frequently in the primary and age related forms of amyloidosis, less frequently in transthyretin familial amyloidosis, and only rarely in secondary forms of amyloidosis
- Cardiac infiltration results in cardiac symptoms dominated by congestive heart failure, arrhythmias, and cardiomyopathy
- The diagnosis of amyloidosis requires a multidisciplinary approach, including clinical examination, biochemical tests, imaging, and genetic analysis, and should be confirmed by Congo red staining in polarised light of a tissue sample
- In addition, immunohistochemistry should be used to define the protein fibril type because it is of diagnostic, prognostic, and therapeutic importance

CONCLUSIONS

Amyloidosis often affects the cardiovascular system. The heart is usually infiltrated by amyloid fibrils in primary amyloidosis and age related forms of amyloidosis, less commonly in transthyretin familial amyloidosis, and rarely in secondary amyloidosis. Cardiac infiltration results in cardiac symptoms dominated by congestive heart failure and arrhythmias. The diagnosis of amyloidosis requires tissue sample confirmation. Congo red staining in polarised light is the method of choice at the present. However, the pathologist should not only make the generic diagnosis of the presence of amyloid, but should also determine the protein fibril type by means of immunohistochemistry, because it is of diagnostic, prognostic, and therapeutic importance.

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