Reviews in Health Care 2013; 4(4): 231-247

RARE DISEASES

Narrative review

Overview of systemic and localized amyloidosis

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Abstract

Amyloidosis is a family of protein misfolding disorders, in which insoluble fibrillar proteins deposit extracellularly and cause end organ damage. Depending on the precursor protein, clinical manifestations in amyloidosis vary significantly. In systemic amyloidosis, the heart, kidneys, and nerves are most commonly affected, resulting in congestive heart failure, arrhythmia, nephrotic syndrome, renal failure, and peripheral and autonomic neuropathies. In localized amyloidosis, amyloid deposits at the site of production, so only one organ is disrupted. Once amyloidosis is confirmed histologically, the precursor subtype must be identified using immunohistochemistry, immunofixation, electron microscopy, or laser microdissection and mass spectrometry. Treatment should not be initiated prior to the identification of the type of amyloidosis. Currently, treatment focuses on the suppression of the precursor protein: in AL amyloidosis, chemotherapy or autologous stem cell transplants suppress production of immunoglobulin light chains; in AA amyloidosis, anti-microbial and anti-inflammatory agents suppress amyloid A production; and in AF amyloidosis, a liver transplantation removes the source of mutant transthyretin protein production. Newer drugs are being developed to target amyloidosis at an epigenetic level or stabilize folding intermediates, but there are currently in development.

Keywords

Localized amyloidosis; Rare disease; Systemic amyloidosis

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Disclosure
The author declares no conflicts of interest

Introduction

Amyloidosis is a family of protein folding disorders, in which insoluble fibrillar proteins deposit extracellularly, causing organ damage. By definition, these fibrils have a distinct stain to Congo Red. On light microscopy, amyloid deposits have an amorphous, salmon-colored appearance, but give off an apple-green birefringence under polarized light [1]. Greater than 28 precursor proteins have been described in amyloidosis, the most common being immunoglobulin light chain (AL), transthyretin (ATTR), amyloid A (AA), and amyloid β-peptide (Aβ) [2]. The initial step in amyloidogenesis, in which a soluble precursor becomes an unstable fragment, is mediated by genetic mutations, proteolytic events, environmental factors, or excessive concentration of the precursor protein. The unstable fragments form self-aggregating folding intermediates, which become β-pleated sheets and subsequently amyloid fibrils. Serum amyloid P and glycosaminoglycans helps stabilize amyloid fibrils [3]. Amyloid fibrils can cause organ damage by disrupting its architecture or direct cytotoxicity [4]. In localized amyloidosis, the amyloid deposits at the site of production, whereas deposition occurs remotely in systemic amyloidosis. The pattern of organ involvement and clinical manifestations depend largely on the precursor protein, although other factors are involved and are not entirely understood. Currently, treatment of amyloidosis focuses on suppressing production of the precursor protein, although ongoing clinical trials are aiming to stabilize amyloid fibrils. In this review, I will explore the classification, clinical features, diagnosis, and treatment of amyloidosis.

Classification

Amyloidosis falls into two broad categories: systemic and localized (Table 1). The most prevalent and best-elucidated form of localized amyloidosis is Alzheimer's disease. In Alzheimer's disease, Aβ is the major component of the amyloid plaques, which cause synaptic injury, widespread neuronal dysfunction, and neuronal cell death [5]. In localized AL amyloidosis, plasma cells on mucosal surfaces secrete immunoglobulin light chains that misfold and deposit locally, causing obstruction or a mass effect in the tracheobronchial tree, bladder, ureter, or the breast [6-8]. Misfolding of islet amyloid polypeptide or high-dose exogenous insulin can cause amyloid deposition in the pancreas or at the site of administration of insulin [9].

The most common systemic amyloidosis in the developed countries is AL amyloidosis, affecting 6-10 persons/million/year in the United States [10]. AL amyloidosis is a plasma cell dyscrasia in which misfolded immunoglobulin light chains can disrupt the function in most organs, except the brain and vitreous fluid. Specific amino acids in certain loci of the variable domain destabilize the light chain and promote amyloidogenesis [11].

In AA amyloidosis, uncontrolled inflammation or infections lead to the overproduction of amyloid A protein, which is amyloidogenic. Interleukin-1 (IL-1), IL-6 and tumor necrosis factor-α (TNF-α) are the primary inflammatory cytokines that stimulate the release of serum amyloid A. The cause of the underlying inflammation varies largely on geography and access to effective therapies. In developed countries, rheumatologic disorders, primarily rheumatoid arthritis, juvenile rheumatoid arthritis, and ankylosing spondylitis, cause up to 60-75% of AA amyloidosis [12-14]. Other inflammatory processes, such as inflammatory bowel disease and periodic fever syndromes, can cause up from 2-14% of AA amyloidosis, although rates may be higher in countries bordering the Mediterranean Sea [12-15]. Tuberculosis, osteomyelitis, bronchiectasis, recurrent bacteremia from intravenous drug use and other infectious causes are more prominent in the developing world or amongst recent immigrants [15]. With improvement of anti-microbial and immunosuppressive therapy, the underlying cause for inflammation is not always obvious despite a thorough work up. Patients without an identifiable cause of inflammation have more cardiac involvement and may have a worse prognosis [16,17].

	Type	Precursor protein	Organ involvement	Treatment options
Localized	Localized AL	Immunoglobulin light chain	Tracheobronchial, bladder, ureter, breast	Resection, laser excision, conformal radiotherapy
	Alzheimer's disease	Aβ protein precursor	Brain	Acetylcholine esterase inhibitor, NMDA receptor antagonist
Systemic	AL amyloidosis	Immunoglobulin light chain	Heart, kidney, autonomic and peripheral nervous system, GI tract, liver, spleen, pulmonary, endocrine, soft tissue	Chemotherapy, autologous stem cell transplantation
	AA amyloidosis	Serum amyloid A	Kidney, GI tract, spleen, andocrine, rarely heart and autonomic nervous system	Suppressing underling inflammation
	Familiar amyloidosis	Mutant transthyretin	Heart, autonomic and peripheral nervous system, soft tissue	Liver transplantation, investigational drugs (diflunasil, tifamidis, doxycycline, siRNA, ASO)
	Age-realted amyloidosis	Wild-type transthyretin	Heart, soft tissue	Supportive care
	Less common familial amyloidosis	Apolipoprotein A1, apolipoprotein AII, fibrinogen Aα chain, lysozime, gelsolin, cystatin C	Varies based on precursor protein/mutation	Supportive care

Table I. Types of localized and systemic amyloidoses

ASO = Anti-sense deoxynucleotides; siRNA = small interfering RNA

Amyloidosis involving the transthyretin (TTR) can occur as variant (ATTR) or wild-type. Transthyretin protein is a transport protein for thyroid hormone and retinoic acid, although gene-expression profiling suggest an additional role in altering chaperone molecules intracellularly [18]. ATTR, a form of familial amyloidosis, is a family of inherited amyloidosis due to single nucleotide polymorphisms in the transthyretin protein. While >100 have been identified, approximately 80 are pathologic [19]. The most common mutations include isoleucine-122, methionine-30, and alanine-60 and symptom onset and organ involvement can vary significantly, based on the mutation.

Wild-type TTR can aggregate and form amyloidosis in the age-related or senile-systemic amyloidosis (SSA). SSA, a disease of aging, predominantly affects men (50:1 male:female expression) and deposits exclusively the heart [20]. Clinically significant congestive heart failure from cardiac amyloidosis in SSA can occur in 5.5% to 16% of elderly aged at least 80 years [21,22]. However, autopsy series have detected scattered, clinically irrelevant amyloid deposition in 10-25% over the age of 80. After liver transplantation, case reports have shown accelerated deposition of wild type TTR in the native heart, even at younger ages [23]. The cause of aggregation of wild type TTR has not been elucidated. Variant and wild type TTR can be differentiated only with genotyping.

Other, less frequent forms of amyloidosis have been described. Dialysis-related amyloidosis, due to aggregation of β-2 microglobulin, deposits in the bone or peri-articular tissue. It is less common with modern hemodialysis techniques, in which newer high flux dialysis membranes effectively filter β-2 microglobulin; it can still occur with peritoneal dialysis [24]. Several other rare familial amyloidoses have been described, with mutations genes coding for apolipoproteinA1, apolipoproteinA2, fibrinogen, gelsolin, lysozyme, and cystatin, each with different organ involvement and age of onset based on the precursor type and locus of mutation.

Clinical features

In amyloidosis, multiple organs can be affected and a thorough physical and laboratory examination is necessary to determine the extent of involvement. A systematic assessment may provide clues about the precursor protein and help anticipate and minimized toxicities of treatment (Table II).

Organ-specific clinical features

A cardiac event is the most common cause of death in amyloidosis. Patients with cardiac involvement have rapid and progressive congestive heart failure or arrhythmias. Less frequently, in the setting of amyloid deposition in the small vessels of the heart, patients can have anginal symptoms, but a normal coronary angiogram [25]. On physical examination, patients have signs of right-sided heart failure with bilateral leg edema, elevated jugular venous pressures, and hepatomegaly. Electrocardiography (ECG) can show low-voltage on limb leads with a pseudo-infarct pattern, q waves in the pattern of a coronary artery. This occurs more frequently in AL amyloidosis than SSA [26]. Echocardiography reveals concentric left, and sometimes right, ventricular hypertrophy, an enlarged interventricular septum, and a normal or slightly reduced left ventricular ejection fraction [1]. The interventricular septum is thicker in SSA than AL amyloidosis (17.8 mm vs. 14.3 mm, p=0.002) [20]. A diminished mitral A wave, the result of increased left-sided filing pressures and atrial infiltration by amyloid, increases the risk for atrial thrombosis and systemic embolic disease; systemic anti-coagulation may be warranted

Organ involved	Symptoms	Signs	Other
Heart	Congestive heart failure, angina, arrhythmia	Bilateral leg edema, elevated JVP, hepatomegaly	ECG: low voltage limb leads, pseudo-infarct pattern. Echocardiogram: concentric LVH, increased interventricular septal thickness
Kidney	Leg swelling, abdominal distension	Leg edema, anasarca	Nephritic syndrome
Peripheral neuropathy	Numbness pain	Decreased deep tendon reflexes, loss of vibration sense	
Autonomic neuropathy	Nausea, vomiting, bloating early satiety, colic, dry eye/mouth, erectile dysfunction	Orthostatic hypotension	
Gastrointestinal tract	Malabsorption, hemorrhage, pseudo-obstruction	Cachexia	Positive stool guaics
Liver/spleen	Hepatic/splenic rupture	Hepato-/splenomegaly	Elevated alkaline phosphatase, Howell-Jolly bodies
Pulmonary/ tracheo-bronchial	Hoarseness, recurrent pneumonia, exertional dyspnea	Airway obstruction, pleural effusions	Bronchoscopy: nodular/irregular airway. Pulmonary Function Tests: distal/ proximal obstruction, decreased DLCO. CT: interlobular septal thickening in basilar and peripheral destruction
Soft tissue	Impaired speaking or eating	Macroglossia, submandibular gland enlargement, peri orbital ecchymoses	
Coagulopathy	Thrombosis/bleeding		Low factor X levels

Table II. Clinical Features of amyloidosis

even in the absence of atrial fibrillation [27]. Newer techniques, such as tissue Doppler imaging and strain Doppler imaging, have improved detection of cardiac amyloidosis at an early stage [28]. Cardiac magnetic resonance imaging (MRI) helps differentiate amyloidosis from other forms of hypertrophic cardiomyopathy, with the former showing global late enhancement of the subendocardium [29]. In ATTR, ^{99}mTc-3,3-diphosphono-1,2-propanodicarboxylic acid (^{99}mTc-DPD) scintigraphy can identify early cardiac involvement despite normal ECG or echocardiography, which is particularly important in certain phenotypes in which only the heart is involved. Furthermore, only one-third of patients with AL amyloidosis with cardiac involvement have positive scans, so ^{99}mTc-DPD scintigraphy can sometimes be used to differentiate ATTR from AL amyloidosis [30]. ^{99}mTc-DPD scintigraphy has a role in diagnosis and prognosis of SSA [31].

Serum cardiac biomarkers play a critical role in determining prognosis, especially in AL amyloidosis. In AL amyloidosis, a prognostic model of overall survival using troponin T (TnT), and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) was proposed and showed the median survival in stage I was 26.4 months, compared to 3.5 months in stage III [32]. An updated prognostic model, devised in the current era of novel chemotherapeutic agents and autologous stem cell transplantation, includes serum free light chain (FLC) measurements and demonstrated a wide range of median survival, ranging from 94.1 months in stage I to 5.8 months in stage IV [33]. Serum cardiac biomarkers can also be elevated in SSA and ATTR, but their prognostic role has not been elucidated [26,34].

Amyloidosis, especially AL and AA, can involve the kidneys. Most commonly, amyloid fibrils deposit in the glomeruli, resulting progressive nephrotic syndrome and renal failure, resulting in dialysis-dependence [3]. In AL amyloidosis, the predominant protein excreted is albumin and the amount can exceed 30 grams daily [3]. The resultant hypoalbuminemia can impede effective diuresis. Amyloid deposition in the tubulointerstitium can cause progressive renal failure in the absence of proteinuria, but this process is more indolent. Rare manifestations of amyloid deposition include nephrogenic diabetes insipidus, due to deposition in the peri-collecting duct tissue, and Fanconi's syndrome, due to proximal tubule injury from light chains [35,36].

Amyloid deposition in the nerves can cause both peripheral and autonomic neuropathy. Peripheral neuropathy can present in stocking-glove distribution with loss of vibration or sensation, absence of deep tendon reflexes, and painful or burning dyesthesias. Symptoms of autonomic neuropathy include orthostatic hypotension (74%), gastrointestinal (GI) symptoms with nausea, vomiting, bloating, early satiety, and colic (71%), secretomotor symptoms with dry eyes or dry mouth (54%), and erectile dysfunction (67%) [37]. Autonomic neuropathy rarely occurs in the absence of peripheral neuropathy. Gastrointestinal tract and the reticuloendothelial system can be involved too. Symptoms of GI involvement include malabsorption, pseudo-obstruction, weight loss, and hemorrhaging [1,38]. The presence of GI amyloidosis does not necessarily indicate systemic disease as approximately 20% of cases are due to localized amyloidosis [39]. Amyloid fibrils deposit in the liver, resulting in hepatomegaly, elevated alkaline phosphatase, and rarely spontaneous hepatic rupture [40,41]. Likewise, splenic dysfunction, illustrated by the presence of Howell-Jolly bodies, and spontaneous rupture have been described [42,43].

Pulmonary and airway amyloidosis, which can occur in both systemic and localized disease, typically presents with dyspnea, hemoptysis, exertional dyspnea, or hoarseness. Amyloid can deposit in four different areas of the lung: interstitial, nodular, pleural, and tracheobronchial [6]. Most commonly, submucosal deposition in the tracheobronchial airway results in airway obstruction, recurrent pneumonia, hoarseness, and segmental collapse [8,44]. Imaging and bronchoscopy show nodular and irregular narrowing of the airway. Pulmonary function tests vary depending on location of the amyloid, whether involving the tracheobronchial tree proximally or distally. Second, diffuse interstitial disease presents with functional deterioration, although this decline is more commonly due to cardiac amyloidosis [6]. Chest roentgenography shows a reticuloendothelial pattern; a computed tomography of the

thorax shows interlobular septal thickening in basilar and peripheral distribution [44]. Pulmonary function test reveal impaired gas exchange. Third, amyloid can deposit in the pleura causing recurrent unilateral pleural effusions; bilateral pleural effusions more commonly signify severe cardiac amyloidosis [6]. Last, nodular amyloidosis presents as an amyloidoma, a non-cavitary pulmonary nodule, and is rare.

Soft tissue and endocrine glands can be involved. Adrenal and thyroid infiltration result in adrenal insufficiency and hypothyroidism, respectively. Deposition in the tongue can cause macroglossia, an enlarged tongue with tooth impressions laterally and rigidness or crunchiness to palpation. Macroglossia can be functionally debilitating with impairing mastication, swallowing, and speech. Submandibular enlargement can be prominent on physical exam. Spontaneous peri-orbital ecchymosis or purpura from vascular deposition can be a subtle marker of amyloidosis. Nail dystrophy and hair loss can occur too. Finally, factor X deficiency significantly impairs hemostasis and is pathognomonic for AL amyloidosis.

AL amyloidosis

AL amyloidosis has the widest range of organ involvement and most commonly affects the kidneys (46%) and heart (30%) [1]. Cardiac involvement is associated with shorter survival and increased toxicity to therapy in AL amyloidosis, with 40% dying from heart failure or arrhythmias [45]. In AL amyloidosis, troponin I (TnI), troponin T (TnT), N-terminal prohormone of brain natriuretic peptide (NT-proBNP), and serum free light chains (FLC) are all predictors of survival and should be part of routine work up [32]. Factor X deficiency and macroglossia are almost pathognomonic for AL amyloidosis.

AA amyloidosis

AA amyloidosis most commonly affects the kidneys, but can also involve the GI tract, adrenals and the reticuloendothelial system. Autonomic and cardiac involvement is rare and occurs only late in disease course [13]. Nephrotic syndrome slowly improves with suppression of inflammation, but can deteriorate rapidly when the inflammation returns. Risk factors for poor prognosis included age, hypoalbuminemia, end stage renal failure at baseline, and mean arterial pressure [12,13].

ATTR amyloidosis

The individual mutation in TTR predicts the phenotype in familial amyloidosis, including age of onset, clinical course, and prognosis. Variant TTR follows an autosomal dominant pattern and commonly affects the peripheral nervous system with or without heart involvement. Vitreous involvement occurs almost only in variant TTR. The most prevalent mutation is the valine-to-methionine substitution at position 30 (Met-30 or V30M). Arising predominantly in Japan, Portugal, or Sweden, Met-30 follows two different patterns. In mutations arising in patients with Japanese or Portuguese ancestry, Met-30 has high penetrance, early age of onset (twenties to early forties), and rapid progression of autonomic- and polyneuropathy. In patients with Swedish ancestry, age of onset is the middle fifties with low penetrance and slow progression [46]. Cardiac disease commonly presents with arrhythmias, rather than heart failure. The threonine-to-alanine substitution at position (Ala-60 or T60A), occurring predominantly in patients with Irish and British ancestry, results in polyneuropathy and cardiac involvement. Cardiac involvement is nearly universal, some series reporting 100% involvement [21]. The most common variant TTR affecting only the heart is the valine-to-isoleucine substitution at position 122 (Ile-122 or V1221). Ile-122 is seen in the Caribbean Islands and among African-Americans in the United States [47]. Genotypically, it is present in 4% in this population, but penetrance is incomplete. Compared to aged-, gender-, and ethnically matched population, Ile-122 is associated with higher frequency of congestive heart failure (38% vs. 15%, relative risk 2.62, p=0.084) and trend towards lower mortality (relative risk 1.46, p=0.08) [48].

Diagnosis

Even with high clinical suspicion, a tissue biopsy is necessary for diagnosis of amyloidosis. On Congo red stain, amyloid appears red on light microscopy, but apple-green under polarized light (Figure 1) [1]. While Congo red stain is the gold standard, this is typically performed only when the pathologist has a high level of suspicion for amyloidosis. Other more commonly performed histological techniques can be suggestive of amyloidosis. On hematoxylin and eosin stain, amyloid appears as a salmon-colored pink, amorphous extracellular substance. Thioflavin T, which is less specific than Congo red, stains amyloid yellow on light microscopy and red with fluorescence [49]. On electron microscopy, amyloid fibrils are non-branching, randomly arranged, and have a 7 to 12 nm diameter and can be diagnostic of amyloidosis [2].

While the affected organ has the highest sensitivity, a biopsy of the abdominal fat pad is safer, less invasive, and commonly the first diagnostic step. A limited amount of abdominal fat is necessary for diagnosis, so a fine needle aspirate is sufficient. The fat pad aspiration has 80-90% sensitivity for AL amyloidosis, but only 65-75% for AA amyloidosis [50]. In localized amyloidosis, amyloid fibrils deposit locally and the fat pad aspirate is negative. If the fat pad aspirate is negative and suspicion for amyloidosis is high, then the involved organ should be biopsied. The sensitivity of a biopsy of an involved heart is 100%, liver is 97%, and kidney is 94% [2]. In familial amyloid polyneuropathy, a sural nerve biopsy has only 33% sensitivity, so a rectal biopsies can be attempted if the nerve biopsy is normal [51].

Once the diagnosis of amyloidosis is confirmed, the precursor protein must be identified prior to starting treatment since treatment will vary based on the type of amyloidosis. For instance, amyloid infiltration of the heart can occur in AL amyloidosis, SSA, or familial amyloidosis due mutant TTR. While ^{99}mTc-DPD scintigraphy, interventricular wall thickness, and cardiac biomarkers can be suggestive of one type of amyloidosis, these tests are not diagnostic. Without identification of the type of amyloidosis, patients can be treated for the inappropriate type of amyloidosis. For example, an elderly patient with SSA and an MGUS, both diseases with an increased prevalence in the elderly, are sometimes inappropriately treated with a systemic chemotherapy, even though both diseases should only be observed. To prevent any incorrect therapy, the precursor protein should be identified using any available involved tissue, including the fat pad.

It is important to note that the presence of a hypertrophic cardiomyopathy in systemic amyloidosis is not necessarily diagnostic of cardiac amyloidosis. The differential for left ventricular hypertrophy

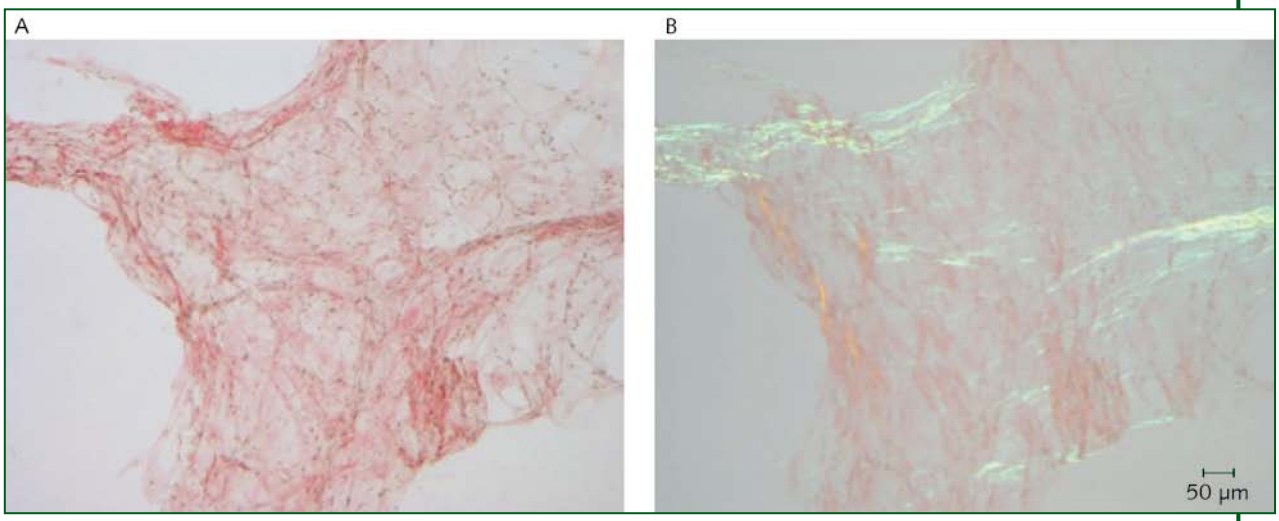

Figure 1. Amyloid on Congo red: red on light microscopy (A) and apple-green under polarized light (B)

includes hypertensive cardiomyopathy, hypertrophic obstructive cardiomyopathy, and other rare infiltrate cardiomyopathies. Hypertensive heart disease can be differentiated from amyloid cardiomyopathy by increased or normal voltage on ECG in the former. Echocardiography can also differentiate amyloidosis from other hypertrophic cardiomyopathy by diffusely increased echogeneitic and increased interatrial septal thickness. An endomyocardial biopsy is the gold standard to differentiate hypertrophic cardiomyopathies, but should only be performed if it will change clinical decision making.

The first step in typing the amyloid is immunofluorescence and immunohistochemistry using antibodies against immunoglobulin light chains, TTR, and amyloid A. Due to the nature of amyloid, even the commercially available antibodies can be inaccurate. For instance, the variability in light chains or structural changes in variant TTR may impair antibody interaction with the epitope, yielding a false negative. Second, charge interactions between amyloid and the reagent can cause false positives, especially in AA [2]. To reduce background and non-specific staining, immunofluorescence is preferred over immunohistochemistry, but requires fresh or paraffin-embedded tissue.

If the subtype is still not identified, immunogold electron microscopy or laser microdissection and mass spectrometry should be performed. In immunogold electron microscopy, gold-labeled antibodies directed again common subtypes stain tissue sections. This technique allows visual confirmation that the gold-labeled antibodies bind the amyloid fibril, allowing exclusion of non-specific binding to the background. However, it has the aforementioned limitations related to the antibodies.

Laser microdissection and mass spectrometry is becoming increasingly important in typing amyloidosis. Amyloid deposits are identified with Congo red, dissected under microscopy, digested with tryptic peptides, then analyzed by liquid chromatography mass spectroscopy [52]. The initial series found 98-100% sensitivity and specificity to detect the most common amyloidosis types. Subsequent series have described accurate detection of rare forms of amyloidosis [53]. Thus, mass spectrometry is becoming the gold standard due to its high sensitivity and specificity, as well as its ability to identify rare forms of amyloidosis. Current barriers to widespread use including the technical expertise and cost.

If TTR is detected, differentiating variant from wild-type form is necessary and is achieved in two steps: isoelectric focusing and genotyping. Isoelectric focusing, a process of separating variant from wild type TTR using gel electrophoresis, has 96% sensitivity and 100% specificity [54]. In SSA, only one band is present; in ATTR, two bands are present: variant and wild type. If variant TTR is demonstrated, genotyping of the TTR gene is necessary to determine the mutation.

Scintigraphy with radioisotope labeled serum amyloid P component (SAP) can visualize amyloid deposits in most organs, but it has limited benefit. Serum amyloid P, which along with glycosaminoglycans helps stabilize amyloid fibrils, is universally present in all amyloidosis, irrespective of the subtype. The burden of amyloid deposits can be reproducibly quantified with SAP, although clinically relevant organ involvement can be easily determined with physical exam and laboratory findings. While detection of heart involvement certainly alters treatment options, cardiac MRI is more sensitive than SAP, which cannot reliably image the heart [2].

Treatment

Current therapies suppress or stabilize of the precursor protein formation and interfere with fibrillogenesis. In AL amyloidosis, chemotherapy with or without autologous stem cell transplantation can be curative. In AA, treatment focuses on stopping the underlying cause of inflammation. In ATTR amyloidosis, orthotopic liver transplantation is curative. For SSA and other less common amyloidoses, treatment is typically supportive.

AL amyloidosis

Chemotherapy strategies in AL amyloidosis are usually extrapolated from management of multiple myeloma, but the toxicities are typically more significant in amyloidosis. Treatment strategies remain controversial, with some advocating for high dose chemotherapy with autologous stem cell transplantation, while others recommend standard dose chemotherapy only. One randomized, phase III clinical trial compared standard dose melphalan with dexamethasone and high-dose melphalan with autologous stem cell transplantation (HDM/SCT) [55]. Although this was designed as a non-inferiority trial, the standard dose melphalan arm had longer survival (56.9 vs. 22.2 months, p=0.04). This should be interpreted with caution, since the HDM/SCT transplant arm had 26% treatment-related mortality and an addition 20% died prior to receiving the stem cell infusion. With appropriate patient selection, institutional experience, and improved anti-microbials, the treatment-related mortality can be reduced to 1-5% [56,57]. While upfront morbidity and mortality are undeniable, long term remission can be achieved; median OS in all patients treated HDM/SCT was 6.3 years, up to 13.2 years in patients who had a hematologic complete response [56]. Given the potential for cure, patients with AL amyloidosis should be evaluated by an experienced center for HDM/SCT.

AL amyloidosis is currently undergoing a treatment renaissance with the advent of novel agents. Chemotherapy typically consists of two or three drugs, almost universally including corticosteroids. While these new drugs are efficacious, toxicities are more severe in amyloidosis and initial doses are typically lower than in other hematologic malignances. Even corticosteroids can result in significant volume retention in patients with cardiac or renal involvement. Dexamethasone and the alkylating agent melphalan have formed the core of treatment until recently, resulting in response rates up to 67% and median OS to 5.1 years [58,59]. More recently, the combination of the proteosome inhibitor bortezomib and dexamethasone has become widely used, due to the tolerability and rapid onset of action. A recent Phase 1/2 trial showed that time to first and best response was 0.7 and 1.2 months, respectively, with a twice-weekly regimen [60]. Significant toxicity, dose reduction, and discontinuation of bortezomib can be reduced with once weekly dosing. If a rapid response is necessary due to rapidly progressing involvement of a critical organ, one strategy is to initiate twice weekly dosing and reduce to once weekly when a satisfactory response is achieved.

Immunomodulators have had variable success in AL amyloidosis. Thalidomide is rarely used and is poorly tolerated due to bradycardia, dyspnea, edema, and fatigue. Two prospective studies showed Grade 3 or 4 toxicities in up to 65% of patients and up to half withdrew due to toxicity [61,62]. The second-generation immunomodulator lenalidomide is better tolerated, but patients with chronic renal insufficiency or cardiac involvement particularly suffer grade 3 to 4 toxicity in 50-75% of patients [63,64]. With a reduced dose, response rates are as high as 47% and median progression free survival is 4.2 years. One prospective study evaluated the third generation pomalidomide and results are promising. In a pre-treated cohort, almost 50% had a hematologic response, only 9% withdrew from the study due to toxicity, and the one-year progression free survival was 59% [65].

Triplet chemotherapy is also showing promise in AL amyloidosis. Cyclophosphamide, bortezomib, and dexamethasone had a 94% hematologic response rate and improved functional capacity in some patients, allowing them to undergo HDM/SCT. Despite multi-organ disease and 59% (n=10) having advanced cardiac amyloidosis, the regimen was remarkable well tolerated [66]. Caution should be used interpreting this data since this was a small, retrospective series with inherent selection bias and variable entry criteria. The combination of bortezomib, melphalan, and dexamethasone has been studied in prospectively, although the results are available in abstract form only [67]. Again, the hematologic response rate was 94%, but 75% had grade 3/4 thrombocytopenia and required dose-adjustments. Long-term survival data are incomplete, so triplet chemotherapy cannot yet be considered a standard of care. Nonetheless, incredible progress has been made over the last decade in both standard dose chemotherapy and autologous stem cell transplantation.

AA amyloidosis

Serum amyloid A production is driven by inflammation, so treatment of AA amyloidosis should focus on controlling the underlying disease. The earliest successful example was treatment of familial Mediterranean fever (FMF) with colchicine to prevent AA amyloidosis. Although the pathophysiology of FMF is not clear, colchicine can prevent attacks [68]. Compared to non-compliance, the adherence to prophylactic colchicine reduced the incidence of AA amyloidosis from 30% (16/54) to 0.4% (4/960) [69]. Suppression of inflammation from many underlying diseases has caused regression of AA amyloidosis in multiple case reports and small series. In rheumatoid arthritis, initiation of biological therapies can reduce in serum amyloid A and proteinuria, and improve kidney function [70]. Resection of localized Castleman's disease has resulted in regression of amyloid deposition [71,72].

In AA amyloidosis, an investigational drug targets fibril formation. Eprodisate is a negatively charged, sulfonated molecule that interferes with the interaction between glycosaminoglycans and amyloidogenic proteins, inhibiting formation of amyloid fibrils. In a double-blinded, randomized trial, the eprodisate had a trend towards preventing worsening renal disease ($p=0.06$) and decreasing the rate of decline in creatinine clearance, but did not prevent progression to end-stage renal disease or death [73]. The Food and Drug Administration (FDA) did not approve this medication, thus a new clinical trial (NCT01215747) is accruing patients.

ATTR amyloidosis

Since the majority of TTR protein is produced in the liver, orthotopic liver transplantation (OLT) has become the standard of care for some familial amyloidoses. An OLT replaces production of variant TTR with non-amyloidogenic wild type TTR, theoretically preventing the progression of familial amyloidosis. Nearly 2,000 OLT have been performed worldwide, primarily in patients with V30M [21]. One single-center study showed survival advantage with OLT compared to supportive care at 10 years (83% and 62%) and 15 years (60% vs. 19%, $p<0.001$) [74]. Despite an OLT, progressive amyloid cardiomyopathy and neuropathy can occur in a subset of transplanted patients [21,75]. The new fibrils depositing in the heart are wild type TTR, with previously deposited variant TTR acting as a catalyst for fibrillogenesis.

While OLT can be curative, it requires identification of appropriate donors, availability of a liver, major surgery, and long-term immunosuppression. Therefore, other therapies are necessary. The rate-limiting step in TTR fibrillogenesis is TTR dissociation and monomer misfolding. Diflunisal, an FDA-approved non-steroidal anti-inflammatory drug, binds the thyroxine binding site on TTR, stabilizing the tetramer, raising the activation energy for dissociation, and inhibiting fibrillogenesis [76]. Pharmacokinetics suggests that adequate serum concentrations can be reached to stabilize TTR [76]. A single-arm, open label Phase I/II study showed that diflunisal can be given safely in cardiac amyloidosis [77]. A randomized Phase 3 trial (NCT00294671) to determine the effect of diflunisal on progression of neuropathies has finished accrual and results should be available soon. A second small molecule tafamidis also stabilizes the TTR tetramer through interaction with the thyroxine-binding site. A randomized, placebo-controlled, double-blinded study did not show a statistically significant improvement in neuropathic symptoms by intention-to-treat analysis. Given the higher-than-anticipated liver transplantation dropout rate, efficacy evaluable analysis was performed and showed that tafamidis improved neuropathic scores and a significant delay in neurologic progression [78]. Importantly, it stabilizes not only the most common forms variant TTR, but also wild type TTR [79]. Unlike the European Medicines Agency that approved the drug, the FDA requested additional studies and a second trial (NCT01435655) is currently recruiting patients. Doxycycline is another small molecule that is undergoing investigation in amyloidosis. In a mouse-model for TTR and AL amyloidosis, doxycycline inhibited amyloid formation [80,81]. A phase II, open-label study of doxycycline with tauroursodeoxy-

cholic acid showed an acceptable safety profile and stable disease for one year in a 20 patient cohort [82]. Additional phase II trials are accruing patients. Last, newer techniques are targeting expression of TTR on a genetic level. In a transgenic mouse model, antisense oligonucleotides can suppress TTR levels by 80% [83]. Industry sponsored Phase II/III trials for small interfering RNA (NCT01617967) and antisense oligodeoxynucleotides (NCT01737398) are or soon will recruit patients [21].

Localized AL amyloidosis

Treatment of localized amyloidosis consists of local therapy to resect amyloid deposits or ablate the plasma cells. Of the localized AL amyloidoses, tracheobronchial airway has the strongest data. Laser excision is currently the standard of care, since it not only removes the involved tissue, but may also kill the plasma cells producing the light chains. Therapy needs to be repeated frequently, requiring 2 to 4.7 treatments annually, depending on the location in the airway [6]. Eventually, scar tissue forms in the airway and patients develop untreatable, progressive respiratory compromise and survival a median of 9 years after diagnosis [8]. Conformal radiotherapy has shown benefit in a retrospective review in progressive airway amyloidosis, with prevention of additional deposition in 8/10 patients and improved functional capacity without late morbidity [84]. In cases of diffuse lung involvement from aggressive, localized amyloidosis, corticosteroids were not effective and systemic chemotherapy can rarely be considered [6]. Other localized amyloidoses are also treated with local therapy. Bladder amyloidosis can be treated with transurethral resection, although some cases require a cystectomy and urinary diversion [85]. Breast amyloidosis, which presents with calcification on mammography, requires local excision to exclude malignancy, even with recurrence. In a small series of 7 patients, one had an invasive ductal adenocarcinoma in addition to amyloidosis [7].

Supportive Care

Since amyloidosis is frequently not curable, supportive care is necessary to prolong life and improve its quality. Cardiac amyloidosis causes significant morbidity, so management of volume status and dysrhythmias is critical and difficult. The combination of loop diuretics and spironolactone is effective in managing the volume status, although low dose metolazone may be necessary up to 3 times weekly. Volume overload and subsequent poor cardiac output can result in renal failure, making diuresis harder. Conversely, overdiuresis, especially with metolazone, can rapidly cause orthostatic hypotension and renal failure. Cardiac amyloidosis causes diastolic dysfunction, so a compensatory sinus tachycardia should not be blunted [21]. While low doses of β-blocker can be beneficial by increasing the time in diastole and improving filling and cardiac output, calcium channel blockers and digoxin are an absolute contra-indication. Both can bind amyloid fibrils, the former reducing stroke volume and exacerbating congestive heart failure and the latter increasing the risk of digoxin toxicity. Low dose angiotensin-converting enzyme inhibitors and angiotensin receptor blockers can cause severe hypotension, especially in patients with autonomic nervous system involvement [86]. Atrial fibrillation are best managed with low dose β-blockade (up to 100 mg of metoprolol daily) and amiodarone [21]. Orthotropic heart transplantation (OHT) has an established role in ATTR and has emerging data in AL amyloidosis [21,87]. One retrospective series showed that in patients with severe cardiac involvement that prohibited SCT, OHT followed by HDM/SCT is feasible and may lead to a survival benefit [87].

Irrespective of the precursor protein, untreated renal amyloidosis will eventually progress to end stage renal disease (ESRD). While suppression of the precursor protein can improve the proteinuria and stabilize renal function, patients may nonetheless progress to ESRD [70-72,88]. In amyloidosis, renal transplantation can be safely performed after successfully suppressing the precursor protein. In AL amyloidosis, one small series showed that there was no graft failure due to recurrent amyloidosis at a median follow up of 5 years [89]. In AA amyloidosis, recurrent amyloidosis nephropathy recur-

red in 14% of patients, especially in those continued underlying inflammation [90]. Patients with ESRD from amyloidosis can be evaluated for kidney transplant, especially if the precursor protein is controlled.

Treatment of peripheral and autonomic neuropathies in amyloidosis is similar to management of other neuropathies. Midodrine is effective in increasing standing blood pressure and should be given three times in the morning, noon, and late afternoon. This can be used in symptomatic patients or in anticipation of hypotension from diuresis. Fludrocortisone is a second line therapy, but can aggravate fluid retention. Early satiety can be improved with small, frequent meals. Reglan can be helpful with gastroparesis. Loperamide, atropine/diphenoxylate, octreotide, and tincture of opium can control diarrhea. Gabapentin, pregabalin, or opiate analgesia can be used to manage painful dyesthesias.

Conclusion

In summary, amyloidosis is a family of protein misfolding disorders, defined by extracellular deposition of a fibrillar protein that stains with Congo red. Due to its rarity and non-specific symptoms, diagnosis is commonly delayed and organ dysfunction can be significant at diagnosis. Advancements in histology and proteomics have significantly improved the ability to identify the precursor protein, but clinicians and pathologist must consider amyloidosis on their differential for the appropriate tests to be ordered. Treatment of systemic amyloidosis and suppression of the precursor protein has changed dramatically over the last decade. Newer anti-plasma cell, antimicrobial, and anti-inflammatory agents have improved outcomes in AL and AA amyloidosis, but treatment of familial amyloidosis is undergoing the most exciting changes: small molecules that stabilize TTR or affect TTR production at an epigenetic level.

Questions for further research

Currently, treatment of amyloidosis focuses on suppression of the precursor proteins, although some clinical trials are now attempting to stabilize the folding intermediates to reduce deposition of amyloidosis. Future studies in AL amyloidosis should compare the long-term survival in patients treated with novel agents and autologous stem cell transplantation. A second major research focus should attempt to resorb the deposited amyloid fibrils, in hopes of reversing end-organ damage. This could ultimately decrease the morbidity of treatment and improve the quality of life after suppression of the precursor proteins.

The review in brief	
Clinical question	Amyloidosis is a family of rare, progressive, multisystem diseases, in which a significant delay in diagnosis of classification can be fatal. This review attempts to increase the awareness of and review the classification, clinical presentation, diagnosis, and treatment of localized and systemic amyloidosis
Type of review	Narrative
Conclusions	Recent improvements with immunogold electron microscopy and laser microdissection and mass spectrometry have improved the classification of amyloidosis. Newer anti-plasma cell, antimicrobial, anti-inflammatory agents, and small molecules have improved outcomes in systemic amyloidosis
Limitations	This is a brief overview of several distinct diseases, unified by formation of amyloid fibrils. The patient characteristics, natural history, and treatment of each subtype can differ significantly, allowing for only an overview, rather than an in depth review

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