CHAPTER 29

The Amyloidoses

PASHA SARRAF, MD, PHD
JONATHAN KAY, MD

- The prevalence of amyloid diseases varies in different geographic areas. Alzheimer's disease is the most common form of localized amyloidosis, while AL amyloidosis is the most common systemic form of amyloidosis in the United States, while AA amyloidosis is the most common form worldwide.
- Amyloid fibrils resemble a woven cable and are deposited in tissues depending of the specific causative protein. Amyloid deposits do not cause an inflammatory reaction, but interfere with the function of surrounding tissues.
- Clinical manifestations vary according to the type of amyloid disease.
- Diagnosis is made by recognition of deposits with a characteristic "apple green" birefringence under polarizing microscopy and the specific subunit protein by immunohistochemistry.
- Treatment is directed toward reducing production of aberrant proteins or surgical removal of amyloid deposits or affected organs.

The amyloid diseases involve a wide variety of proteins that share in common the property of forming fibrils (1). Deposition and accumulation of these fibrils in various tissues, ultimately interfering with normal organ function, results in the clinical entity known as amyloidosis. The amyloidoses can occur either as isolated localized processes or as systemic disorders involving multiple organ systems. Furthermore, amyloidosis may occur as a primary disease process or as a secondary consequence of other diseases. Both light and heavy chain (formerly known as primary) amyloidosis and familial amyloidosis belong to the former group, whereas serum amyloid A protein and beta$_2$-microglobulin (dialysis-associated) amyloidosis belong to the latter group. In the United States, primary (idiopathic) amyloidosis is the most common form of amyloid disease, while serum amyloid A-associated amyloidosis occurs more commonly in other countries. Alzheimer's disease and prion deposition disease are the only forms of localized amyloid fibril deposition which often lead to serious illness; other forms of localized amyloid deposition usually lead only to mechanical interference and generally are considered to be benign.

The name *amyloidosis* is preserved in deference to Rudolph Virchow, who first used histochemical stains in 1854 to characterize amyloid deposits in pathologic specimens of brain (2). Whereas all other structures in his brain sections stained yellow after the application of iodine and sulfuric acid, the corpora amylacea stained pale blue with iodine and brilliant violet upon subsequent acidification. Because this pattern of staining was characteristic of plant cellulose, Virchow concluded that the corpora amylacea was composed of a celluloselike substance that he labeled "amyloid." The term *amyloid*, derived from the Greek word *amylon*, refers to materials that contain or resemble starch. This is a misnomer, however, as it now is known that amyloid deposits contain mainly protein, even though some carbohydrate-containing substances may associate with the proteins. The study of amyloid has focused mainly on its protein composition.

The understanding of the protein structure of amyloid has been advanced by the observation that Congo red, an aniline textile dye, stains amyloid deposits. Congo red molecules deposit and align perpendicular to the long axis of amyloid fibrils, markedly enhancing the anisotropy of these fibrils and appearing as "apple green" birefringence under polarizing microscopy. Independent of their protein of origin, by definition, all amyloid proteins share three common characteristics: a cross-beta-pleated sheeted structure, an organized fibrillar ultrastructure, and congophilia producing "apple green" birefringence.

Amyloid deposits are amorphous and consist of a number of components (3). The wide array of proteins that may result in amyloidosis are not derived from common precursors and do not have any particular genetic homology. However, each protein, in the proper setting, can form amyloid fibrils. Other components of amyloid deposits include the serum amyloid P compo-

nent and proteoglycans which, although not part of the fibrils themselves, may contribute to amyloid formation in situ.

Amyloid nomenclature is based on the name of the primary subunit protein: all amyloid fibril proteins are named "protein A-" with a suffix that identifies the specific subunit protein (e.g., protein AL for light chain amyloidosis and protein AA for serum amyloid A amyloidosis). Both the amyloid type and the resulting disease are named for the protein. Thus, *AA amyloidosis* replaces the label *secondary amyloidosis*, and *AL amyloidosis* replaces the names *primary amyloidosis* and *myeloma-associated amyloidosis*. Currently, 24 amyloid proteins that can cause clinically apparent amyloidosis have been identified. However, most of these types of amyloidosis are quite rare and occur primarily as hereditary diseases with clustering of cases in families.

PATHOGENESIS

The structure of an amyloid fibril resembles that of a woven cable, in which three to six filaments wrap around one another to form a fibril (4). Individual filaments have a lamellar beta sheet structure that is composed of thousands of individual, noncovalently associated peptide subunits. This higher ordered structure facilitates the binding of certain small molecules, such as Congo red, and macromolecules, such as proteoglycans and serum amyloid P component.

The initiation and progression of amyloidogenesis is entirely dependent on the causative protein, but generally follows one of three pathogenetic processes: overproduction and deposition of wild-type protein, deposition of a mutated variant of a protein, or deposition of protein fragments that have been generated by aberrant endoproteolytic cleavage. The first scenario results in AA or senile ATTR amyloidosis, in which serum amyloid A protein or transthyretin is overproduced and deposited. The second scenario, in which a mutation destabilizes the wild-type protein and confers amyloidogenic properties on the new variant protein, results in hereditary types of amyloidosis such as familial ATTR or AGel amyloidosis. The third scenario is illustrated by AL amyloidosis, in which normal immunoglobulin light chains undergo limited proteolysis that yields the amyloidogenic form. The unfolded proteolytic cleavage products then self-associate by a mechanism known as *seeded polymerization* to form a superstructure called a *seed*, the formation of which is rate-limiting. Once produced, the seed acts as a template for the rapid addition of new monomers, thus accelerating the assembly of an amyloid fibril.

Amyloid fibrillogenesis can occur both in vivo and in vitro. Virtually any protein can form amyloid fibrils in vitro, but only a limited repertoire of molecules form detectable deposits of amyloid in vivo. The reasons for this discrepancy are unknown, but likely are related to modifying influences of the affected individual. These include, but are not limited to, the presence and specificity of endoproteolytic enzymes, the presence of inherited single nucleotide polymorphisms that counteract the effect of the amyloidogenic mutation, and the amount of amyloidogenic protein synthesized. Factors such as these determine the timing of disease onset and the rapidity of its progression.

The presence of detectable amyloid is the sine qua non for expression of disease in patients. Although the extent and rapidity of organ damage and disease expression varies between patients, even in those with similar types of amyloid proteins, the whole body burden of amyloid correlates directly with the extent of disease. Thus, reducing the total amount of amyloid may stabilize or improve clinical manifestations of disease.

Amyloid deposits do not elicit a significant inflammatory reaction in vivo. This is consistent with the observation that amyloid fibrils prepared ex vivo do not induce a systemic acute-phase response or an inflammatory reaction when administered to experimental animals. In the absence of significant inflammation, it might be assumed that amyloid fibrils result in clinical disease because of a direct cytotoxic effect on surrounding cells. In Alzheimer's disease, oligomers of amyloid-beta protein interfere with cognition in experimental animals by causing cytopathic changes in neurons. However, there is no evidence that other amyloid proteins are directly cytopathic to surrounding tissues in vivo. Rather, the clinical course of amyloidosis suggests that physical interference of amyloid deposits with normal organ function is the primary mechanism of disease pathogenesis. In cardiac amyloidosis, the intrinsic contractility of heart muscle is not affected by amyloid deposition; instead, amyloid fibril deposition in the myocardium alters the elastic properties of cardiac muscle and causes a restrictive cardiomyopathy with reduced filling. Similarly, retinal cells are not affected by transthyretin deposition in the vitreous humor, as years of blindness can be reversed by replacing the vitreous fluid. Furthermore, patients with familial amyloidosis who undergo organ transplantation to restore function of failing organs do not exhibit recurrent organ dysfunction until many years after transplantation, when amyloid deposits once again become clinically evident. These observations suggest that the clinical manifestations of the amyloid diseases result from interference with normal organ and tissue architecture that causes predictable patterns of progressive organ dysfunction over time. Thus, it is critical to intervene to inhibit amyloid formation and deposition, as quickly as possible, so as to prevent disease progression.

EPIDEMIOLOGY

The prevalence of the amyloid diseases varies in different geographic locations (5). Although in the United States and throughout the world, Alzheimer's disease is the most frequent form of amyloidosis, this chapter will focus primarily on the most commonly encountered systemic forms of the disease. In the United States, AL is the most common form of systemic amyloidosis. The stable population of Olmstead County, Minnesota, has provided reliable data regarding the disease prevalence between 1950 and 1989, suggesting that approximately 1 in 100,000 people will develop AL amyloidosis (6). Of the Mayo Clinic patients with AL amyloidosis, 18% had a diagnosis of multiple myeloma and 16% had a prior diagnosis of monoclonal gammopathy of unknown significance. Among all patients with multiple myeloma, approximately 20% will develop AL amyloidosis.

Worldwide, AA is the most common form of systemic amyloidosis. In industrialized countries, inflammatory conditions are the leading cause of AA amyloidosis, whereas systemic or chronic infections are responsible for the majority of cases of AA amyloidosis in developing countries.

Of the hereditary forms of amyloidosis, those due to transthyretin (TTR) mutations occur most frequently, causing either systemic ATTR amyloidosis or isolated senile cardiac amyloidosis. The TTR mutation V122I is the most common amyloid-associated TTR variant worldwide and occurs in 3.9% of African Americans and in over 5% of the population in some areas of Western Africa. TTR V122I is the most frequently identified subunit protein in patients with isolated senile cardiac amyloidosis.

CLINICAL FEATURES

Amyloidosis can present as either a systemic or a localized disease. There are four classes of systemic amyloidosis: AL, AA, ATTR, and $A\beta_2M$ (Table 29-1). Numerous forms of localized amyloidosis have been identified. Alzheimer's disease and isolated laryngeal and urinary tract amyloid deposits are the most common forms of localized amyloidosis.

With the exception of Alzheimer's disease, where direct cellular cytotoxicity in the brain is observed, the clinical presentation of the other amyloidoses is caused by mechanical disruption of normal physiologic function as previously described. The clinical presentation of amyloidosis depends on the amyloid subunit protein involved. Table 29-2 summarizes the amyloid proteins, their associated clinical syndromes, and the distribution of organ involvement in the localized amyloid diseases. In the following sections, the most common acquired systemic amyloid diseases (AL, AA, ATTR, and $A\beta_2M$) are reviewed.

TABLE 29-1. SYSTEMIC AMYLOIDOSIS.

		HC	IMMUNOHISTOLOGY				
TYPE	ASSOCIATION	CR	SAP	λ/κ	SAA	β_2M	TTR
AL	Plasma cell dyscrasia	+	+	+	–	–	–
AA	Chronic inflammation	+	+	–	+	–	–
$A\beta_2M$	Chronic dialysis	+	+	–	–	+	–
ATTR	Familial	+	+	–	–	–	+

ABBREVIATIONS: λ/κ, lambda and kappa light chains; β_2M, beta$_2$-microglobulin; CR, Congo red; HC, histochemistry; SAA, serum amyloid A protein; SAP, serum amyloid P component; TTR, transthyretin.
This table illustrates the histochemical and immunohistochemical properties of the main systemic amyloidoses. Tissue may be stained with Congo red (CR) to demonstrate presence of amyloid deposits and may subsequently be immunostained for the specific amyloid subunit protein to determine the specific type of the amyloid deposit.
Listing of the human amyloidoses and their precursor proteins. The amyloid diseases and their subunit proteins have been divided broadly into systemic and localized disease. It should be noted that some of the proteins may have overlapping disease presentations: for example, AL amyloidosis may present in a localized form, as well as in a systemic form (see text), and AGel amyloidosis may present in a systemic form as a neuropathy, as well as in a localized form. This has been indicated by "L" (localized) or "S" (systemic) under the heading: "Main Clinical Setting."

AL Amyloidosis

The clinical manifestations of AL amyloidosis are protean (7). The kidney, heart, and liver are the organs that are most frequently and most prominently involved; however, all organs other than the central nervous system may be affected. In the kidney, AL amyloid deposits primarily in the glomerulus, causing nephrotic syndrome that usually manifests as proteinuria with initial daily urine protein excretion of more than 2 g. Not infrequently, in more advanced disease, daily urine protein excretion may be as high as 5 to 15 g.

Cardiac involvement develops insidiously. By the time most patients with AL amyloidosis present with clinically apparent cardiac disease secondary to amyloidosis, significant myocardial damage already has occurred. Supraventricular tachyarrhythmias may occur as a result of atrial enlargement. The restrictive cardiomyopathy can result in significant orthostatic hypotension due to restricted ventricular filling, compounded by autonomic dysfunction caused by peripheral nervous system involvement.

Bleeding and motility disorders are the most common presentations of AL amyloid deposition in the gastrointestinal tract. Early satiety, caused by delayed gastric

TABLE 29-2. HUMAN AMYLOIDOSIS: PROTEINS, PRECURSORS, AND DISEASE.

	TYPE	FIBRIL PROTEIN	MAIN CLINICAL SETTING
Systemic	Acquired	AL, Ig light chain	Plasma cell disorder
		AH, Ig heavy chain	Plasma cell disorder
		ATTR, transthyretin	Familial amyloidosis
		AA, (apo) serum amyloid A	Inflammation associated
		Aβ_2M, beta$_2$-microglobulin	Dialysis associated
	Hereditary	AFib, Fibrinogen alpha chain	Familial systemic amyloidosis
		AAPOAI, Apolipoprotein AI	Familial systemic amyloidosis
		AAPOAII, Apolipoprotein AII	Familial systemic amyloidosis
		ALys, Lysozyme	Familial systemic amyloidosis
Localized	CNS	Aβ, beta-protein precursor	Alzheimer's, Down's
		APrP, Prion disease	Creutzfeldt–Jacob disease, Gertsmann–Straussler–Scheinker disease
		ACys, Cystatin C	Herediatry cerebral hemorrhage with amyloidosis (Icelandic)
		ABri ABriPP precursor protein	Familial dementia (British/Danish)
	Ocular	AGel, Gelsolin	Familial amyloidosis (Finnish)
		ALac, Lactoferrin	Familial corneal amyloidosis
		AKer, Keratoepithelin	Familial corneal amyloidosis
	Endocrine-related	ACal, (pro) Calcitonin	Medullary thyroid carcinoma
		AIAPP, Amylin (islet amyloid polypeptide)	Insulinoma, type 2 diabetes
		AIns, insulin	Insulinoma, type 2 diabetes
		APro, Prolactin	Pituitary amyloid
		AANF, Atrial natriuretic factor	Cardiac/atrial amyloid
	Other	AKep, Keratin (keratoepithelin)	Cutaneous amyloidosis
		AMed, Medin (lactadherin)	Aortic amyloidosis in the elderly

emptying, is also a frequent symptom. Bacterial overgrowth with significant malabsorption may cause diarrhea and may result in deficiencies of vitamin B_{12}, folic acid, and carotene deficiency. Hemorrhage may occur in any part of the gastrointestinal tract, although the stomach and small intestine are more frequently affected. AL amyloid often deposits in the liver, although it rarely produces symptoms.

Peripheral nervous system involvement, which can occur months to years before visceral involvement, occurs in as many as 20% of patients with AL amyloidosis. It may manifest as either or both a sensorimotor neuropathy and an autonomic neuropathy. Paresthesias develop first in the lower extremities and, over time, may extend proximally. Motor nerve involvement, although rare, may be severe and may result in foot drop and gait abnormalities. Autonomic neuropathy occurs commonly in patients with AL amyloidosis, often resulting in gastrointestinal dysmotility, impotence, and orthostatic hypotension.

There are two predominant pulmonary manifestations of AL amyloidosis. Occasionally, AL amyloid may present as a tumorlike mass in the lung parenchyma, often with concomitant enlargement of hilar and peritracheal lymph nodes. Although these masses may enlarge progressively, they generally are not life-threatening. Alternatively, there may be diffuse interstitial infiltration of the lung parenchyma, causing stiffness of the lungs and restrictive pulmonary physiology. Infrequently, AL amyloid may deposit locally in laryngeal and tracheal tissue, resulting in hoarseness and, sometimes, significant upper airway obstruction.

Hematological abnormalities of AL amyloidosis include purpura and thrombosis. Amyloid infiltration of blood vessel walls causes the vessels to become fragile. Disruption of cutaneous capillaries results in extravasation of red blood cells and purpura. In a patient with AL amyloidosis, periorbital purpura can result from maneuvers as innocuous as rubbing the eyes or keeping the head facing downwards for a prolonged period, leading to the characteristic "raccoon eyes" appearance. Factor X deficiency occurs in this disorder and is believed to result from absorption of this clotting factor by large amyloid deposits in the spleen, as well as from protein loss in the setting of nephrotic syndrome. This, in addition to abnormalities in the plasminogen system, results in an increased incidence of venous thrombosis.

Although AL amyloidosis is the most prevalent form of amyloidosis that involves skin, skeletal muscle, and the tongue, its soft tissue and articular manifestations occur infrequently. Carpal tunnel syndrome, often bilateral, may be caused by amyloid deposits in the wrist compressing the median nerve and may occur years before the full clinical presentation of systemic disease. Amyloid infiltration of skeletal muscle, typically involv-

ing the tendons and capsular structures of the shoulders, may result in pseudohypertrophy ("shoulder pad sign") in a patient who is otherwise very cachectic. Amyloid deposits in bone, such as in the femoral neck, may appear as cystic lucencies on radiographs and may compromise the tensile strength of bone, resulting in pathologic fractures. Although uncommon, macroglossia may develop in patients with AL amyloidosis. The enlarged tongue is firm to palpation and may cause problems with speech and deglutition and produce the sensation of choking.

AL amyloidosis results from the abnormal and clonal expansion of B-cell lymphocytes. However, although necessary for the elaboration of this disease, it is not sufficient to have monoclonal cell expansion and light or heavy chain synthesis. AL amyloidosis may develop in the setting of Waldenstrom's macroglobulinemia, multiple myeloma, monoclonal gammopathy of unknown significance, or benign B-cell expansion. The amount of protein produced by these clones seems unimportant, as between 10% and 20% of patients with AL amyloidosis have no monoclonal protein detectable in serum or urine. The primary structure of light chains appears to be particularly important to the development of this condition because normal serum ratios of light chains are reversed and lambda chains are found in AL amyloid deposits far more frequently than are kappa chains. Certain lambda chain subtypes have a greater propensity to form fibrillar deposits than do others. Furthermore, AL amyloid fibril subunit proteins almost always contain the variable segment (V_L) of the immunoglobulin light chain, either in its entirety or as a segment. However, the reasons for selective organ involvement and for differential rates of disease progression among affected individuals remain unclear.

AL amyloidosis is the most serious of the amyloid diseases, with survival following tissue diagnosis of only 18 to 24 months (8,9). An initial presentation with carpal tunnel syndrome or peripheral neuropathy frequently connotes a better prognosis than does an initial presentation with cardiac involvement. Multiple myeloma may appear after the diagnosis of AL amyloidosis in a small proportion of patients, emphasizing the importance of continued follow-up and appropriate testing.

The treatment of AL amyloidosis is directed towards reducing the aberrant plasma cell clone with medications, such as melphalan and prednisone (8,9). Occasionally, chemotherapeutic agents, such as cyclophosphamide or chlorambucil, are also used. Vinca alkaloids or adriamycin should be used with great caution, as they may be especially toxic to those patients who have established neuropathy or cardiomyopathy. For eligible patients, high-dose intravenous melphalan with autologous stem cell transplantation is the treatment of choice (10). In patients with more advanced disease, intermediate-dose melphalan with stem cell rescue may provide a better tolerated alternative therapy (10). Among patients who are eligible for and receive treatment with bone marrow transplantation, mean survival is extended to 40 months, whereas it remains 18 months in those patients who are ineligible for transplantation.

AA Amyloidosis

Serum amyloid A (SAA) protein is a member of the acute-phase protein family (11). The normal plasma concentrations of SAA range between 1 and 3 μg/mL. During an acute-phase response, concentrations rapidly increase 200- to 300-fold and, upon its resolution, return to the normal range within days. Amyloidosis due to SAA is the most common form of systemic amyloidosis worldwide. Any inflammatory stimulus that turns on the SAA gene can induce AA amyloidosis. The most common cause of AA amyloidosis is tuberculosis but, in industrialized nations, rheumatologic diseases such as rheumatoid arthritis, juvenile rheumatoid arthritis, spondyloarthritides, and autoinflammatory syndromes are the leading causes of AA amyloidosis. AA amyloid fibrils may be detected in biopsy specimens from asymptomatic individuals, preceding any signs of systemic amyloidosis by many years.

The most important manifestation of AA amyloidosis is renal disease, which generally presents as nephrotic syndrome. This may occur after 10 to 20 years of inflammatory arthritis and may occur in patients in whom the underlying primary inflammatory disease has subsided. Thus, AA amyloidosis may be confused with the presentation of other pathologic processes involving the kidney, such as gold-induced nephropathy. Furthermore, acute inflammatory triggers may accelerate the occurrence of systemic AA amyloidosis in patients who previously have been exposed to inflammatory insults, such as tuberculosis or other chronic infections. Therefore, nephrotic syndrome may develop over the course of several weeks in patients with new active tuberculosis, perhaps because preexisting foci of localized amyloid deposits may accelerate the progression of systemic AA amyloidosis.

Gastrointestinal hemorrhage may occur in patients with AA amyloid. Deposition of protein AA along blood vessel walls leads to decreased distensibility and increased fragility, with episodic vessel rupture and bleeding. Although described in the literature, clinically significant involvement of the heart, nerves, skeletal muscle, or tongue is very unusual in AA amyloidosis. It is important to consider the diagnosis of AA amyloidosis in patients who present with nephrotic syndrome, even in those with no history of an inflammatory or infectious disease. This presentation occurs in patients

with familial Mediterranean fever (FMF) who have subclinical elevation of SAA and other acute-phase reactants, but who are otherwise asymptomatic. These patients eventually may progress to systemic amyloidosis. Because many of these patients with FMF have lived in developing countries, environmental exposures, such as endemic infections, may potentiate this presentation by causing chronic inflammation, thereby increasing their risk of developing AA amyloidosis.

Serum amyloid A, a highly conserved acute-phase protein, is produced primarily in the liver. There are four SAA genes: *saa1* and *saa2* are under acute physiologic regulation, *saa3* is a pseudogene and is not expressed, and *saa4* is expressed at a continuous basal level. Although each SAA protein has been identified as an apolipoprotein component of the high-density lipoprotein (HDL) particle, only the SAA4 protein appears to function in this capacity, under physiological conditions, and does not appear to be involved in the pathogenesis of amyloid disease. SAA1 and SAA2, which are mainly responsible for the pathogenesis of AA amyloidosis, also can be found in the HDL particle at low levels. However, serum levels of both of these proteins increase dramatically in response to glucocorticoids and to proinflammatory cytokines, such as interleukin 1 and interleukin 6. Therefore, the broad conservation of these proteins across species and the tightly regulated acute control of their expression suggest a more important role for these SAA proteins in the control of inflammation. Thus, it is thought that chronic inflammation results in continuous overproduction of these proteins, which, over time, lead to form amyloid fibrils and deposit in tissues.

Full-length SAA1 and SAA2, each 104 amino acids, are cleaved at their N-termini by endoproteases in the liver to produce fragments of 76 amino acids. Both the full-length proteins and the cleaved products are found in serum and in AA amyloid deposits. In AA amyloidosis, both SAA1 and SAA2 can form amyloid fibrils; however, SAA1 usually contributes much more to fibril formation than does SAA2. The reason for this is unclear. Furthermore, the *saa1* gene has single nucleotide polymorphisms that define three haplotypes: 1.1 (1.alpha), 1.2 (1.beta), and 1.3 (1.gamma). Caucasian patients with the 1.1/1.1 (1.alpha/1.alpha) genotype have a three- to sevenfold increased risk of developing AA amyloidosis (12).

Treatment of AA amyloidosis is directed towards controlling the underlying inflammatory disease process. The clinical outcome of AA amyloidosis is more favorable when the serum SAA concentration remains below 10 mg/L (11). For more advanced disease, renal transplantation effectively restores renal function in patients with AA amyloidosis. However, unless the underlying inflammatory process is suppressed, AA amyloid may deposit in the transplanted kidney.

ATTR Amyloidosis

The hereditary amyloidoses are caused by a variety of unrelated proteins (Table 29-2). These syndromes exhibit autosomal dominant inheritance with varying degrees of penetrance. Even though the gene mutation is present at birth, clinical features of disease usually do not manifest until after the third decade of life. These syndromes share clinical features, typically presenting with cardiomyopathy, nephropathy, and polyneuropathy. However, each amyloidogenic protein should be considered to cause a discrete disease entity, with other unique clinical features. The vast majority of the hereditary amyloidoses are caused by deposition of transthyretin (TTR) variants, with more than 100 mutations having been identified (13). TTR is also known as prealbumin, because it runs faster than does albumin on gel electropheresis. Transthyretin is a plasma protein that carries about 20% of thyroxine in plasma, as well as vitamin A associated with retinol binding protein. TTR is synthesized in the liver as a single polypeptide and, in plasma, forms a tetramer that consists of four identical monomers. The wild-type protein has significant beta sheet structure; a single amino acid substitution contributes to its aggregation and fibril formation.

Not all TTR-related amyloidosis is due to mutations in TTR. Fragments of wild-type TTR may form amyloid fibrils that deposit in the heart, causing senile cardiac amyloidosis. This nonheritable disorder affects about 25% of individuals over the age of 80 years.

Most TTR-related amyloidoses present initially with peripheral neuropathy. This often is a sensorimotor neuropathy involving the distal lower extremities that progresses proximally to involve the proximal extremities. Carpal tunnel syndrome may be the initial presentation in 20% of cases, with ATTR amyloid deposits compressing the median nerve. Autonomic neuropathy can cause gastrointestinal symptoms, such as alternating constipation and diarrhea, or genitourinary symptoms, such as incontinence or impotence.

Although peripheral nervous system disease is associated with significant morbidity, cardiomyopathy and renal disease are the predominant causes of mortality among patients with ATTR amyloidosis. The majority (60%) of deaths are due to cardiomyopathy, whereas renal disease accounts for only 5% to 7% of deaths. Vitreous amyloid deposits occur in 20% of patients with ATTR amyloidosis. These are thought to arise from TTR that is secreted by the choroid plexus and that forms amyloid fibrils, which accumulate in the vitreous.

ATTR amyloidosis is diagnosed by using genetic techniques to identify TTR mutations: the majority of mutations in ATTR occur in exons 2 through 4. The use of polymerase chain reactions to identify restriction fragment length polymorphisms has become commonplace to diagnose affected patients and to

identify carriers of the mutant gene among their family members.

ATTR amyloidosis is treated by replacing the liver and other affected organs. Liver transplantation results in synthesis of wild-type (normal) TTR, with rapid disappearance of the variant transthyretin from the circulation. Combined liver/kidney transplantation has been performed in patients with ATTR amyloidosis with significant renal involvement. In patients affected by ATTR amyloidosis, it is important to intervene before severe malnutrition or cardiomyopathy develops, because transplant survival declines rapidly in those affected individuals. Amyloid deposition may continue, even after organ transplantation, perhaps related to the presence of small deposits of abnormal protein that serve as a nidus for the subsequent deposition of normal proteins. Because of this, patients with earlier presentations of ATTR amyloidosis may require repeat organ transplantation.

$A\beta_2M$ Amyloidosis

$A\beta_2M$ amyloid deposits predominantly in osteoarticular tissue (14). The presence of shoulder pain, carpal tunnel syndrome, and irreducible flexion contractures of the fingers in a patient undergoing long-term hemodialysis is highly suggestive of $A\beta_2M$ ($beta_2$-microglobulin or dialysis-related) amyloidosis. Signs and symptoms of $A\beta_2M$ amyloidosis are infrequently observed in patients with chronic renal failure who have not yet received dialysis treatment.

Axial skeletal involvement, which occurs in about 10% of patients undergoing long-term hemodialysis, presents as a destructive spondyloarthropathy, the radiographic features of which include narrowing of the intervertebral disk spaces and erosion of the vertebral endplates without appreciable formation of osteophyte. The lower cervical spine is most often affected; however, similar changes may also occur in the dorsal and lumbar spine. Cystic deposits of $A\beta_2M$ amyloid within the odontoid process and the vertebral bodies of the upper cervical spine and peri-odontoid soft tissue masses of $A\beta_2M$ amyloid, termed *pseudotumors*, have also been demonstrated. Although neurologic compromise occurs infrequently, significant myelopathy has resulted from $A\beta_2M$ amyloid deposits in the cervical and lumbar spinal canal, especially in patients who have received hemodialysis for 20 years or longer.

Cystic bone lesions may develop in the appendicular skeleton of patients undergoing long-term hemodialysis. Subchondral amyloid cysts, most commonly found in the carpal bones, may also occur in the acetabulum and in long bones, such as the femoral head or neck, the humeral head, the distal radius, and the tibial plateau. Unlike brown tumors of hyperparathyroidism, these bone cysts typically occur adjacent to joints and increase in number and enlarge with time. Pathologic fractures, especially of the femoral neck, may occur through areas of bone weakened by amyloid deposits.

Visceral deposits of $A\beta_2M$ amyloid also have been identified in patients receiving long-term dialysis, most for 10 years or longer. Although gastrointestinal tract and cardiovascular complications have been reported, visceral $A\beta_2M$ amyloid deposits usually do not cause symptoms.

$Beta_2$-microglobulin, the subunit protein in $A\beta_2M$ amyloidosis, is the light chain of class I major histocompatibility antigens. Normally present in most biologic fluids, it is filtered by glomeruli and catabolized after proximal tubular reabsorption. Because the rate of $beta_2$-microglobulin synthesis exceeds the rate of its removal by different dialysis modalities, serum $beta_2$-microglobulin levels are elevated up to 60-fold in patients undergoing dialysis.

Current theories regarding the pathogenesis of $A\beta_2M$ amyloidosis implicate the role of advanced glycation end product (AGE) modification of proteins, which confers on the proteins resistance to proteolysis, increased affinity for collagen, and the ability to stimulate activated mononuclear leukocytes to release proinflammatory cytokines such as tumor necrosis factor alpha (TNF-alpha), interleukin 1 beta (IL-1 beta), and interleukin 6 (IL-6). AGE-modified proteins are poorly cleared by dialysis modalities (14,15). Thus, patients undergoing dialysis have elevated levels of these modified proteins, as compared with individuals with normal renal function or functioning renal allografts. AGE-modified $beta_2$-microglobulin has been identified in amyloid deposits of patients receiving long-term hemodialysis and may play a significant role in the development of $A\beta_2M$ amyloidosis. The propensity for $A\beta_2M$ amyloid to deposit in osteoarticular tissue may be due to the enhanced binding of AGE-modified proteins to collagen. Surgery may be necessary for symptomatic patients with large deposits of $A\beta_2M$ amyloid. Over the past decade, hemodialysis with newer, more permeable membranes appears to have postponed the onset of carpal tunnel syndrome and bone cysts and reduced the incidence of $A\beta_2M$ amyloidosis. $A\beta_2M$ amyloid deposits do not progress and may regress in patients who have undergone successful renal transplantation. Patients with $A\beta_2M$ amyloidosis who undergo successful renal transplantation experience a marked reduction in joint pain and stiffness. Thus, early renal transplantation in appropriate candidates, before significant $A\beta_2M$ amyloid deposition has occurred, may be the most effective preventive measure currently available for this condition.

Localized Amyloidosis

The localized forms of amyloidosis can involve many organ systems including the eye, the genitourinary tract,

the endocrine system, and the respiratory tract (Table 29-2). With the exception of Alzheimer's disease, these types of amyloidosis are rare and challenging to diagnose. The pathophysiologic principles governing disease expression in the localized forms of amyloidosis are similar to those for the systemic forms. The most frequently occurring forms of localized amyloidosis affect the genitourinary and respiratory tracts (13).

Localized genitourinary amyloidosis may involve the entire tract, but more frequently involves the bladder and urethra and causes hematuria or signs of obstruction. The responsible amyloid subunit protein often is immunoglobulin light or heavy chain, but occasionally may be SAA. The localized finding of AL or AH amyloid deposits may prompt an exhaustive search for systemic disease, often with negative results. However, localized AL or AH amyloidosis usually is self-limited and does not portend a severe prognosis. Treatment consists of excision of the localized amyloid deposits.

In the respiratory tract, AL amyloid deposition often produces localized forms of disease. Three forms of localized amyloidosis affect the respiratory tract: tracheobronchial amyloidosis, which accounts for half of cases, nodular parenchymal amyloidosis, which accounts for about 45% of cases, and diffuse parenchymal amyloidosis, which accounts for about 5% of cases. In tracheobronchial amyloidosis, there is either localized or diffuse involvement of the tracheobronchial tree with submucosal deposition of amyloid. Computed tomography (CT) scanning demonstrates nodules or plaques of amyloid, sometimes with calcification, or circumferential thickening of the trachea, main bronchus, and lobar or segmental bronchi with luminal narrowing. In nodular parenchymal amyloidosis, CT scanning demonstrates nodules with sharp and lobulated margins in a peripheral, subpleural location. The nodules vary in size from micronodular to up to 15 cm in diameter; calcification is seen in half of the cases. In diffuse parenchymal or alveolar septal amyloidosis, amyloid deposition is widespread, involving both small vessels and the parenchymal interstitium; multifocal small nodules of amyloid also may be present. High-resolution CT scanning demonstrates abnormal reticular opacities, interlobular septal thickening, small (2–4 mm diameter) nodules, and confluent consolidated opacities predominantly in the subpleural regions (16). This pattern of localized amyloidosis sometimes is indistinguishable from systemic amyloidosis. Patients with this form of diffuse parenchymal pulmonary amyloidosis are more likely to die from respiratory failure than are patients with tracheobronchial or nodular parenchymal amyloidosis..

Localized amyloid deposition limited to the respiratory tract may be excised to cure this form of limited amyloidosis. Other amyloid subunit proteins may also deposit in the respiratory tract, but these are rare and generally do not result in significant pathology.

DIAGNOSIS

Imaging

Scintigraphy using ^{123}I-serum amyloid P component has been used to identify the systemic distribution of amyloid deposits (17). Serial imaging has demonstrated both progression and regression of amyloid deposition. However, this technique is limited in that it exposes patients to radioactive allogeneic protein and it is availability only in specialized centers.

The only widely available imaging technique that yields information specific for the diagnosis of systemic amyloidosis is echocardiography (18,19). Specific echocardiographic features of amyloidosis include atrial enlargement, reduced left ventricular size, biventricular and atrial septal thickening, and increased myocardial echogenicity. In more advanced disease, restrictive filling patterns become more evident. Unfortunately, by the time a patient has echocardiographic manifestations of amyloidosis, median survival is only 6 months. Also, echocardiography does not demonstrate regression of amyloid disease, even after successful therapy.

Cardiac magnetic resonance imaging (MRI) is a rapidly advancing field and has supplemented echocardiography in the diagnosis of cardiac amyloidosis (20). Cardiac MRI, using gadolinium enhancement, provides high spatial resolution (approximately 2 mm) and tissue contrast, differentiating diseased tissue from normal myocardium. In patients with amyloid involving the heart, cardiac MRI demonstrates qualitative global and subendocardial enhancement after intravenous administration of gadolinium. Although there are no sine qua non MRI findings for cardiac amyloidosis, future studies may elucidate a combination of noninvasive tools that could be used to select patients for definitive, more invasive, endomyocardial biopsy, as well as to follow the natural history of cardiac amyloid disease.

Because there are no findings specific for systemic amyloidosis, imaging should be used as an adjunct to physical examination and appropriate laboratory testing to evaluate symptomatic patients. Although the gastrointestinal tract is almost always involved in systemic amyloidosis, radiographic manifestations of gastrointestinal amyloidosis are infrequently seen. Ischemia and resulting edema, due to amyloid deposition in vessels, may cause symmetrical thickening of mucosal folds that appear as target lesions on abdominal CT scanning.

Ultrasound or CT scanning may reveal enlargement of the kidneys in early stages of amyloidosis. Ultrasonography usually demonstrates diffusely increased echogenicity of the renal parenchyma with preservation of the corticomedullary contrast, because the cortical architecture remains grossly normal in early disease. As disease progresses, the kidneys may appear contracted with substantial cortical thinning.

Histopathology

Once amyloidosis is suspected, its diagnosis is confirmed by a biopsy that demonstrates the characteristic "apple green" birefringence under polarizing microscopy and the specific subunit protein by immunohistochemistry. The biopsy may be taken from either an involved or an uninvolved organ. The latter approach usually is preferred because of the high risk of complications and discomfort associated with biopsy of internal organs. Three types of screening biopsies typically are performed to diagnose amyloidosis: gastrointestinal tract (rectal or gastroduodenal) biopsy, subcutaneous abdominal fat aspiration, and labial salivary gland biopsy (5).

Rectal biopsy, performed by sigmoidoscopy or proctoscopy, is the preferred gastrointestinal tract biopsy because of the accessibility of this site (5). The biopsy specimen must include submucosal blood vessels, which are more likely to contain amyloid deposits than are those in the mucosa or muscularis layers. Although the data are most robust for rectal biopsy, gastric or duodenal biopsies can be diagnostic of amyloidosis if the tissue specimen contains blood vessels of the appropriate size.

Abdominal fat aspiration was first performed after it was observed that specimens taken at autopsy of patients with amyloidosis often contained amyloid deposits around adipocytes; the highest density of amyloid deposits was in the adipose tissues isolated from the scalp and the abdominal wall (21). The reported sensitivity of abdominal fat aspiration varies between 55% and 75%, but is similar to that of rectal biopsy. This technique is useful for the diagnosis of AA, AL, and ATTR amyloidosis; however, because of the limited organ distribution of $A\beta_2M$ amyloid deposits, abdominal fat aspiration cannot reliably be used to diagnose $A\beta_2M$ amyloidosis.

Labial salivary gland biopsy samples the accessory salivary glands in the labial mucosa (22). Previously, gingival biopsy had been used to detect amyloid deposition, but was found to be of low sensitivity. In AA, ATTR, and AL amyloidosis, labial salivary gland biopsy has sensitivity comparable to that of rectal biopsy or abdominal fat aspiration.

If the suspicion for amyloidosis is high and one of the above techniques does not yield a positive finding, an involved organ should be biopsied. In the setting of renal involvement, a kidney biopsy usually provides diagnostic information. The organs predominantly involved in ATTR and AL amyloidosis, the heart and the bone marrow, should be biopsied in these forms of the amyloidosis to confirm the diagnosis. Although the sural nerve may be involved in the amyloid diseases, sural nerve biopsy is less desirable because it usually is painful and slow to heal and may leave a residual sensory deficit. Furthermore, the patchy distribution of amyloid deposits renders sural nerve biopsy less sensitive than biopsy of other involved organs.

Three points are essential to consider in the diagnosis of the amyloid diseases (23). (1) The pretest probability of a biopsy for amyloid is determined by the patient's clinical presentation. To determine pretest probability, it is critical to consider the patient's history (including a thorough family history), in the context of a complete physical examination and laboratory evaluation that includes a serum and urine protein electrophoresis and urinalysis to assess for proteinuria. (2) Immunohistochemistry should always be performed on tissue specimens that are being evaluated for amyloid deposits to identify the specific amyloid subunit protein. Occasionally, a patient with an inflammatory disorder may develop AL disease or a patient with a serum monoclonal protein may develop AA amyloidosis. Because the treatment of these diseases differ dramatically, it is imperative to establish a definite diagnosis. (3) Deposits of AA amyloid in abdominal fat are not uncommon in patients with inflammatory diseases, such as rheumatoid arthritis or ankylosing spondylitis. However, even after long-term follow-up, most of these patients do not have evidence of organ dysfunction. Thus, not all patients who have AA amyloid deposits have AA amyloid disease; biopsy results should be interpreted with caution.

SUMMARY

Rheumatologists play an important role in the recognition and treatment of the amyloid diseases. The amyloidoses occur and progress insidiously, taking years to cause clinically apparent organ damage. Delay in diagnosis may result in an increased burden of amyloid fibrils and may reduce the opportunity to effectively deliver therapy and improve prognosis. Each of the amyloidoses presents with a unique constellation of findings which, although not pathognomonic, may lead an astute physician to follow appropriate diagnostic algorithms and intervene expeditiously in the disease process. Finally, although the amyloid diseases are very heterogeneous, the clinical approach to their diagnosis and treatment is straightforward and an earnest understanding of the pathogenetic principles that contribute to disease expression should guide the rational management of the patient.

REFERENCES

1. Sipe JD, Cohen AS. Review: history of the amyloid fibril. J Struct Biol 2000;130:88–98.
2. Majno G, Joris I. Extracellular pathology. In: Cells, tissues, and disease: principles of general pathology. Oxford: Oxford University Press; 2004:250–267.

3. Pepys MB. Amyloidosis. Annu Rev Med 2006;57:223–241.
4. Merlini G, Bellotti V. Mechanisms of disease: molecular mechanisms of amyloidosis. N Engl J Med 2003;349: 583–596.
5. Buxbaum J. The amyloidoses. In: Klippel JH, Dieppe PA, eds. Rheumatology. Mosby yearbook. 1998;8.27.1–8.27.10.
6. Kyle RA, Linos A, Beard CM, et al. Incidence and natural history of primary systemic amyloidosis in Olmsted County, Minnesota, 1950 through 1989. Blood 1992;79: 1817–1822.
7. Kyle RA, Gertz MA. Primary systemic amyloidosis: clinical and laboratory features in 474 cases. Semin Hematol 1995;32:45–59.
8. Skinner M, Anderson J, Simms R, et al. Treatment of 100 patients with primary amyloidosis: a randomized trial of melphalan, prednisone, and colchicine versus colchicine only. Am J Med 1996;100:290–298.
9. Kyle RA, Gertz MA, Greipp PR, et al. A trial of three regimens for primary amyloidosis: colchicine alone, melphalan and prednisone, and melphalan, prednisone, and colchicine. N Engl J Med 1997;336:1202–1207.
10. Comenzo RL. Amyloidosis. Curr Treat Options Oncol 2006;7:225–236.
11. Gillmore JD, Lovat LB, Persey MR, Pepys MB, Hawkins PN. Amyloid load and clinical outcome in AA amyloidosis in relation to circulating concentration of serum amyloid A protein. Lancet 2001;358:24–29.
12. Booth DR, Booth SE, Gillmore JD, Hawkins PN, Pepys MB. SAA1 alleles as risk factors in reactive systemic AA amyloidosis. Amyloid 1998;5:262–265.
13. Benson MD. Amyloidosis. In: Koopman WJ, Moreland LW, eds. Arthritis and allied conditions: a textbook of rheumatology. Philadelphia: Lippincott Williams & Wilkins; 2005:1933–1960.
14. Kay J. β_2-microglobulin amyloidosis. Int J Exp Clin Invest 1997;4:187–211.
15. Miyata T, Inagi R, Iida Y, et al. Involvement of beta 2-microglobulin modified with advanced glycation end products in the pathogenesis of hemodialysis-associated amyloidosis. Induction of human monocyte chemotaxis and macrophage secretion of tumor necrosis factor-alpha and interleukin-1. J Clin Invest 1994;93:521–528.
16. Georgiades CS, Neyman EG, Barish MA, Fishman EK. Amyloidosis: review and CT manifestations. Radiographics 2004;24:405–416.
17. Hawkins PN, Lavender JP, Pepys MB. Evaluation of systemic amyloidosis by scintigraphy with 123I-labeled serum amyloid P component. N Engl J Med 1990;323:508–513.
18. Shah KB, Inoue Y, Mehra MR. Amyloidosis and the heart: a comprehensive review. Arch Intern Med 2006;166: 1805–1813.
19. Falk RH, Plehn JF, Deering T, et al. Sensitivity and specificity of the echocardiographic features of cardiac amyloidosis. Am J Cardiol 1987;59:418–422.
20. Maceira AM, Joshi J, Prasad SK, et al. Cardiovascular magnetic resonance in cardiac amyloidosis. Circulation 2005;111:186–193.
21. Westermark P, Stenkvist B. A new method for the diagnosis of systemic amyloidosis. Arch Intern Med 1973;132: 522–523.
22. Hachulla E, Janin A, Flipo RM, et al. Labial salivary gland biopsy is a reliable test for the diagnosis of primary and secondary amyloidosis. A prospective clinical and immunohistologic study in 59 patients. Arthritis Rheum 1993;36:691–697.
23. Comenzo RL, Zhou P, Fleisher M, Clark B, Teruya-Feldstein J. Seeking confidence in the diagnosis of systemic AL (Ig light-chain) amyloidosis: patients can have both monoclonal gammopathies and hereditary amyloid proteins. Blood 2006;107:3489–3491.