Amyloidosis

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Amyloidosis is a generic term for a group of diseases caused by misfolding and extracellular accumulation of certain proteins as fibrillar deposits that stain with Congo red and produce pathognomonic green birefringence when viewed by microscopy under crossed polarised light. The process of amyloid formation and deposition causes progressive organ dysfunction. Amyloidosis is remarkably diverse and can be hereditary or acquired, localised or systemic and lethal or merely an incidental finding. So far, 27 different human proteins with in vivo amyloidogenic potential have been identified of which 15 cause systemic amyloidosis. The classification of amyloid is based on the fibril protein, and different amyloidogenic proteins give rise to distinct but frequently overlapping clinical syndromes. The kidneys are frequently involved in systemic amyloidosis (Table 29.1) which, without treatment, is usually fatal. Current management of amyloidosis is dependent upon determining the fibril protein and reducing its abundance. This can result in regression of amyloid deposits, prevention or recovery of organ failure and improved survival.

Aetiology and Pathogenesis

Amyloid formation occurs when a protein or peptide loses, or fails to acquire, its physiologic, functional folding and, in its misfolded state, undergoes fibril formation and extracellular deposition. Amyloid deposits display distinctive ultrastructural (beta-sheet conformation) and tinctorial properties.

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The process of amyloid formation and deposition ultimately results in tissue damage and organ dysfunction (Fig. 29.1). The propensity of proteins to form amyloid fibrils in vivo is enhanced by the following:

- A pathologic and sustained increase in concentration of the protein. This is the case of the acute-phase reactant serum amyloid A protein (SAA) in chronic inflammation and of β2-microglobulin in patients with end-stage renal disease (ESRD).
- Presence of an unstable mutant protein, favouring its misfolding and aggregation, as occurs in hereditary amyloidoses.
- Proteolytic remodelling of a protein as in the case of the protease furin cleaving ABri and gelsolin and the β- and γ-secretases releasing amyloid-β (Aβ) peptides.
- Advancing age as in the case of wild-type transthyretin and apolipoprotein A-I both of which have intrinsic amyloidogenic properties and are associated with age-related amyloid deposition.

Frequently, a combination of these factors determines the amyloidogenicity of an individual protein. However,

Table 29.1 Systemic amyloidoses commonly associated with kidney involvement

Amyloid type	Fibril precursor	Note
AL	Light chain V region fragments	Primary, myeloma associated
AA	Serum amyloid A protein (SAA)	Secondary, reactive to chronic inflammation
ALect2	Leukocyte chemotactic factor 2	Sporadic, more common in Mexican Americans and South Asians
AApoAI	Apolipoprotein A-I	Familial
AApoAII	Apolipoprotein A-II	Familial
ALys	Lysozyme	Familial
AFib	Fibrinogen Aα-chain	Familial
AGel	Gelsolin	Familial
ATTR	Transthyretin	Familial, kidney involvement/dysfunction unusual until late stage of disease

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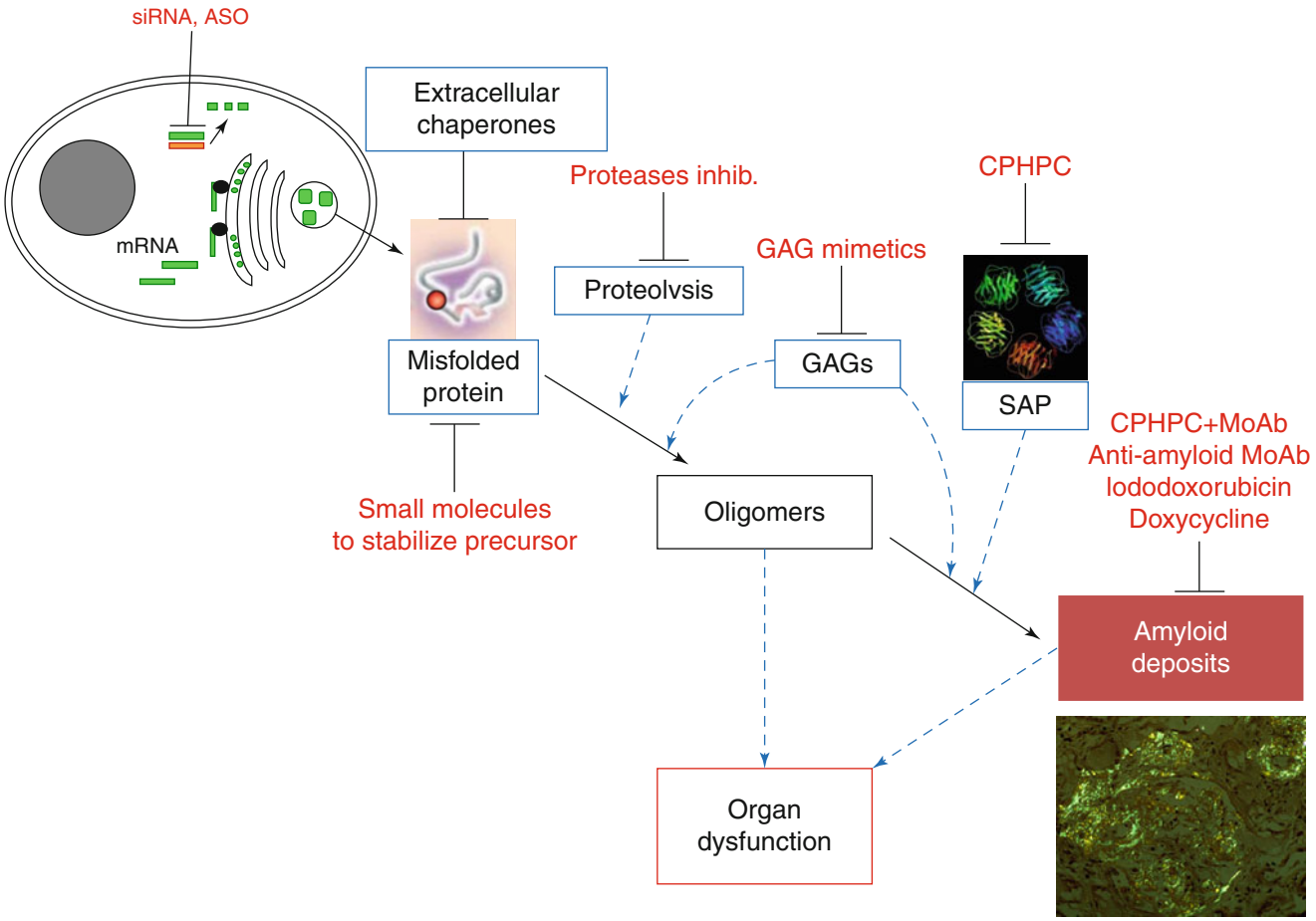

Fig. 29.1 Molecular events leading to amyloidosis. Interaction of the misfolded protein with the extracellular environment may result in proteolytic cleavage and binding to matrix components such as glycosaminoglycans (*GAGs*) and collagen that facilitate aggregation. Several lines of evidence support a role for extracellular chaperones in the in vivo clearance of aggregation-prone extracellular proteins. Serum amyloid P component (*SAP*) binds to amyloid fibrils and protects them from reabsorption. The organ dysfunction may result from the combined action of the cytotoxic prefibrillar aggregates and of the amyloid deposits. Several new therapeutic approaches have been recently developed. The synthesis of the amyloid protein can be silenced using RNA interference (*siRNA*) or antisense oligonucleotides (*ASO*). Small molecules capable of stabilising the amyloid precursor and preventing its misfolding and aggregation (diflunisal, tafamidis) are being tested in patients with ATTR amyloidosis. Inhibitors of proteases (secretase) and compounds interfering with the binding of GAGs to amyloid proteins (eprodisate) are being evaluated in trials. SAP can be cleared from amyloid deposits by using small palindromic drugs (CPHPC). The clearance of amyloid deposits can be promoted and accelerated by specific antibodies through passive and active immunotherapy. Small molecules, such as iododoxorubicin and doxycycline, have shown to be able to disrupt the amyloid fibrils and have been tested in clinical trials

the inherent amyloidogenicity of a specific protein, per se, is not sufficient to account for amyloid deposition in vivo. Undetermined environmental and genetic factors must be involved in amyloidogenesis as only a minority of patients with long-lasting inflammation and persistent elevation of SAA levels develop AA amyloidosis, and, similarly, the disease-associated Val30Met mutation of transthyretin shows significant variation in penetrance and clinical presentation among different ethnic groups and geographic areas.

Amyloid Structure

Electron microscopy and X-ray diffraction analysis reveal that amyloid deposits are composed of rigid, non-branching fibrils with an average diameter of 7.5–10 nm and a cross-β super-secondary structure. More recently, refined structural studies of amyloid fibrils by solid-state nuclear magnetic resonance spectroscopy and microcrystals of small amyloid-like peptides by X-ray diffraction analysis have revealed a degree of structural variation [1, 2].

Common Constituents of Amyloid Deposits

Serum amyloid P component, a glycoprotein of the pentraxin family, binds all types of amyloid, independently of the protein of origin, through a specific binding motif, and protects amyloid fibrils from proteolytic degradation. These properties make SAP a means of imaging amyloid deposits and an

ideal therapeutic target. Proteoglycans are also common in amyloid deposits, and heparan sulphate (HS) proteoglycans, in particular, show similar kinetics of tissue deposition to that of fibrillar proteins. HS accelerates the transition of the amyloid protein from the native state into the amyloidogenic partially folded state and promotes the rate of fibril formation of amyloidogenic immunoglobulin light chains (LCs) and of other proteins through selective binding to a basic motif, as shown for SAA, transthyretin and amyloid-beta. Other common elements found in amyloid deposits are components of the extracellular matrix, such as laminin, entactin and collagen IV.

Kinetics of Fibril Formation

In vitro studies have shown that amyloid fibril formation proceeds, in many instances, through a 'nucleated growth' mechanism, which is reminiscent of crystallisation. Starting from a solution of monomeric proteins, there is an initial lag phase; once a critical nucleus has been generated, fibril formation begins and proceeds with very fast kinetics: any amyloidogenic precursor in its aggregation-prone conformation is rapidly incorporated into the growing fibrils [1]. This seeding mechanism has clinical implications, since the process of amyloid clearance, following a response to therapy, usually leaves traces of 'seeds' in tissues which, following a disease relapse, may trigger rapid re-accumulation of amyloid deposits.

Organ Tropism

Amyloid deposition may occur in almost any organ. Nonetheless, specific amyloidogenic proteins tend to deposit predominantly in defined organs, for example, the kidney for fibrinogen Aα-chain and leukocyte chemotactic factor 2, the peripheral nerves for the transthyretin Val30Met variant, and the joints and bones for wild-type β_2-microglobulin. Several factors may contribute to determining the site of amyloid deposition: local protein concentration, interaction with collagen, tissue-specific glycosaminoglycans, pH, specific local proteolytic enzymes or cellular receptors. In AL amyloidosis the physicochemical characteristics (amino acid composition and conformation of the variable region) of the LC may be the most significant factor in determining the type and location of organ dysfunction.

Mechanisms of Tissue Damage

These have not been fully elucidated; the presence of large amounts of amyloid material can disrupt tissue architecture and mechanically interfere with the physiologic function of affected organs [3]. However, compelling evidence suggests that prefibrillar oligomeric species also contribute to organ dysfunction. Prefibrillar oligomers from transthyretin, Aβ, LCs and the prion protein have been shown to be toxic in vitro and/or in vivo. For example, in AL amyloidosis light chains exert a direct cytotoxic effect on cardiomyocytes. Following reduction of the amyloidogenic LC concentration after chemotherapy, heart failure can reverse, with rapid reduction of the serum concentration of the amino terminal fragment of pro-brain natriuretic peptide (NT-proBNP), a marker of cardiac dysfunction, despite unaltered myocardial amyloid load on imaging [4].

Epidemiology

Systemic amyloidosis is a rare disease accounting for approximately 1 in 2,000 deaths in the UK and presumably other developed countries. Although cases of amyloidosis have been reported in children it is predominantly a disease of mid to late life and accounts for 4 % of adult renal biopsies and 1.6 % of patients starting dialysis [5].

Systemic Amyloidosis Associated with Monoclonal LCs, AL Amyloidosis

The age-adjusted incidence of AL amyloidosis in the USA and the UK has been estimated to be between 5.1 and 12.8 per million persons per year, and AL is the diagnosis in 60–70 % of patients with amyloidosis seen at large referral centres. Approximately 60 % of cases are men and median age at presentation is 65 years; it can occur in young adults and is probably under-diagnosed in the elderly among whom monoclonal gammopathies are most prevalent. AL amyloidosis develops in about 2 % of individuals with monoclonal B-cell dyscrasias [6]. The B-cell dyscrasias underlying systemic AL amyloidosis can include almost any clonal proliferation of differentiated B lymphocytes; 94 % have an underlying clone of plasma cells [7, 8]. The clonal cell burden in AL amyloidosis is usually small and the plasma cell proliferation fraction similar to MGUS. Only 10–20 % of patients who are diagnosed with AL amyloidosis meet myeloma criteria [9]. Progression of the underlying monoclonal gammopathy to overt myeloma is rare in systemic AL amyloidosis, which, in part reflects patients' short survival.

Reactive Systemic, AA, Amyloidosis

The exact incidence of AA amyloidosis is unclear, but it accounts for 10–15 % of the cases of amyloidosis seen at major referral centres. It is always a complication of inflammation, and the list of chronic disorders that can

be complicated by AA amyloidosis is summarised in Table 29.2. In industrialised countries, inflammatory arthritides underlie 60 % of cases. The prevalence of AA amyloidosis in patients with chronic arthritides is between 3 and 6 %. For unexplained reasons the incidence of AA amyloid is much lower in the USA than in Europe, and the incidence appears to be falling in Europe. The median latency between onset of inflammation and diagnosis of amyloid is approximately 17 years, but this varies from less than a year to decades. The median age at diagnosis is 50 years, but presentation in childhood, although becoming less common, is still recognised. As with all types of amyloidosis, AA appears slightly commoner in men who account for 56 % of the largest characterised series.

Table 29.2 Inflammatory conditions which have been reported to underlie AA amyloidosis

Chronic inflammatory arthritides
Rheumatoid arthritis
Juvenile inflammatory arthritis
Ankylosing spondylitis
Psoriatic arthropathy
Reiter's syndrome
Adult Still's disease
Vasculitides
Polyarteritis nodosa
Takayasu's arteritis
Behcet's disease
Systemic lupus erythematosis
Giant cell arteritis/Polymyalgia rheumatica
Chronic infections
Bronchiectasis
Chronic cutaneous ulcers
Chronic pyelonephritis
Chronic osteomyelitis
Subacute bacterial endocarditis
Leprosy
Tuberculosis
Whipples disease
Inflammatory bowel disease
Crohn's disease
Ulcerative colitis
Periodic fevers
Familial Mediterranean fever
Cryopyrin-associated periodic syndrome (CAPS)
TNF receptor-associated periodic syndrome (TRAPS)
Mevalonate kinase deficiency (MVK)
Neoplasia
Hodgkin's disease
Renal cell carcinoma
Adenocarcinoma of the lung, gut, urogenital tract
Basal cell carcinoma
Hairy cell leukaemia
Castleman's disease
Hepatic adenoma
Other
IV and subcutaneous drug abuse
Cystic fibrosis
Kartagener's syndrome
Epidermolysis bullosa
Hypogammaglobulinaemia
Cyclic neutropaenia
Common variable immunodeficiency
Hyperimmunoglobulin M syndrome
SAPHO syndrome

Dialysis-Related Amyloidosis (DRA)

β_2-microglobulin amyloidosis occurs in patients who have been on dialysis for more than 6–10 years or very occasionally in individuals with long-standing severe chronic kidney disease. Relatively few patients have been maintained on peritoneal dialysis for the 5–10 years required to develop symptomatic β_2-microglobulin amyloid, but histological studies of early subclinical deposits suggest that the incidence of DRA is similar among patients receiving the two dialysis modalities [10]. β_2-microglobulin amyloid deposits have been reported in 20–30 % of patients within 3 years of commencing dialysis for ESRD [11] but the incidence seems to have fallen by 80 % between the 1980s and 1990s [12].

Hereditary Systemic Amyloidosis

In the UK the prevalence of hereditary non-neuropathic systemic amyloidosis, which typically presents with renal dysfunction, appears to be in the order of 1.5 per million with most patients presenting in their sixth decade.

Leukocyte Chemotactic Factor 2 (LECT2) Amyloidosis

This is thought to account for up to 2.5 % of renal biopsies containing amyloid [13].

Clinical Features

Systemic Amyloidosis Associated with Monoclonal LCs, AL Amyloidosis

The clinical features of AL amyloidosis are protean (86) as any organ other than the central nervous system can be directly involved.

- Many patients present with nonspecific symptoms such as fatigue and weight loss.
- Renal dysfunction is seen in more than 60 % of cases and causes proteinuric renal failure in the context of a normal

or low blood pressure. In recent large studies 44 % of patients presented with chronic kidney disease (CKD) stage 1 or 2 and 16 % with CKD stage 5; median proteinuria was 5–7 g/day and median serum albumin 21–28 g/L [14, 15].

- Cardiac involvement is a major determinant of outcome and occurs in 74 % of patients at presentation, with approximately 30 % presenting with congestive heart failure. Cardiac biomarkers provide a quantitative assessment of cardiac damage (troponin I or T) and wall strain (BNP, NT-proBNP) and are the most important predictors of outcome in amyloidosis [16, 17]. By using the cut-offs of 0.035 mcg/L for troponin T and 332 ng/L for NT-proBNP, patients can be classified into three stages [18], which are useful in selecting therapies and patient stratification in clinical trials.
- Hepatic amyloid is found in 54 % of patients. Despite often substantial hepatomegaly liver function is generally well-preserved with modest elevation of ALP (median of 154 IU/L). Hyperbilirubinaemia is unusual but associated with a poor outcomes and a median survival of 4 months.
- Gut involvement may cause motility disturbances (often secondary to autonomic neuropathy), malabsorption, perforation, haemorrhage or obstruction.
- Painful sensory polyneuropathy with early loss of temperature sensation followed later by motor deficits is seen in 10–20 % of cases and carpal tunnel syndrome in 20 %.
- Autonomic neuropathy leads to orthostatic hypotension, impotence and gastrointestinal disturbances.
- Macroglossia occurs in 10 % and is pathognomonic of AL type (Fig. 29.2).
- Skin involvement is common and usually takes the form of bruising spontaneously or after minor trauma (Fig. 29.3).
- Hyposplenism sometimes causes blood film abnormalities.
- An acquired bleeding diathesis may be associated with deficiency of factor X and factor IX or with increased fibrinolysis.
- Articular amyloid is rare and may superficially resemble acute polyarticular arthritis, or it may present as asymmetrical arthritis affecting the hip or shoulder. Infiltration of the glenohumeral joint and surrounding soft tissues occasionally produces the characteristic 'shoulder pad' sign.

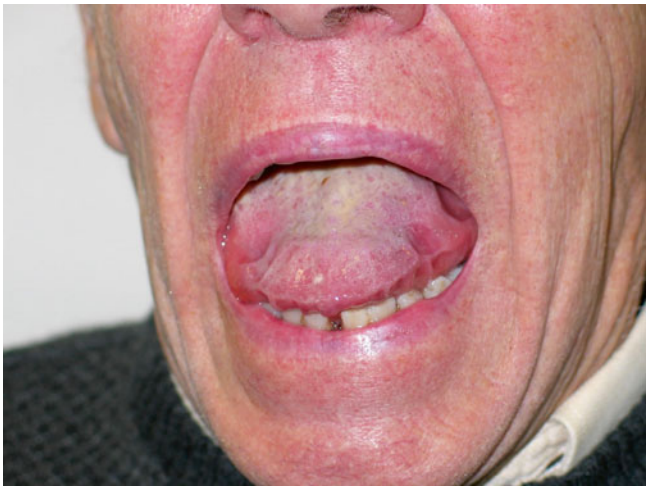

Fig. 29.2 Macroglossia, present in approximately 10 % of cases of AL amyloidosis

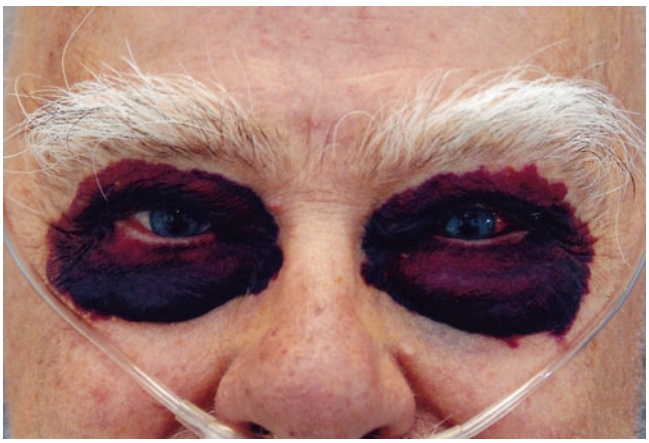

Fig. 29.3 Capillary fragility manifesting as periorbital bruising and conjunctival haemorrhage in AL amyloidosis

Reactive Systemic AA Amyloidosis

The predominant clinical manifestations of AA amyloidosis are renal.

- More than 97 % of patients present with proteinuric kidney dysfunction. Haematuria, tubular defects and diffuse renal calcification occur rarely. Just over 50 % of patients have nephrotic syndrome at presentation. Approximately 10 % of patients are in ESRF at diagnosis and over 40 % eventually progress to ESRF.
- The spleen is almost always infiltrated.
- Adrenal glands are involved in more than 33 %, although clinical hypoadrenalism is rare.
- Hepatosplenomegaly is seen at presentation in 9 % of cases but liver failure is exceptionally rare.
- Malabsorption occurs only in very advanced disease.
- Cardiac amyloidosis is seen in 2 % and only in advanced disease.

Dialysis-Related Amyloidosis (DRA)

β_2-microglobulin amyloidosis is preferentially deposited in articular and periarticular structures, and its manifestations are largely confined to the locomotor system.

- Carpal tunnel syndrome is usually the first clinical manifestation. Some individuals develop symptoms within 3–5 years of initiation of renal replacement therapy, and by 20 years the prevalence was almost 100 %. Older patients appear to be more susceptible to the disease and tend to exhibit symptoms more rapidly [11].
- Amyloid arthropathy tends to occur a little later but eventually affects the most patients on dialysis. It affects the shoulders, knees, wrists and small joints of the hand and is associated with joint swelling, chronic tenosynovitis and, occasionally, haemarthroses. Spondyloarthropathies are also well recognised, as is cervical cord compression. Deposition within the periarticular bone produces typical appearances of subchondral erosions and cysts which can contribute to pathological fractures particularly of the femoral neck, cervical vertebrae and scaphoid.

LECT2 Amyloidosis (ALECT2)

Most patients present in the sixth to seventh decades with slowly progressive renal impairment. Proteinuria tends to be low grade and hypertension is well recognised. Although splenic and adrenal amyloid deposits are visible on SAP imaging, clinically the disease appears to be renal isolated.

Hereditary Non-neuropathic Systemic Amyloidosis

Lysosyme Amyloidosis (ALys)

Most patients present in middle age with proteinuria, very slowly progressive renal impairment and sometimes hepatosplenomegaly with or without purpuric rashes. In retrospect most recollect a long history of dry eyes and dry mouth. Substantial gastrointestinal amyloid deposits are common and important since gastrointestinal haemorrhage or perforation is frequently the cause of death in these patients.

Apolipoprotein A-I Amyloidosis (AApoAI)

Depending on the mutation, patients can present with massive abdominal visceral amyloid involvement, predominant cardiomyopathy or neuropathy. Most patients eventually develop renal failure and despite extensive amyloid deposition, liver function usually remains preserved. Additional features are hypertension, cholestatic hepatopathy and primary hypogonadism with infertility.

Fibrinogen A Alpha Chain Amyloidosis (AFib)

Patients with this form of hereditary amyloidosis frequently do not give a family history of similar disease and are readily misdiagnosed as having AL amyloid. Most patients present in their sixth to seventh decades with proteinuria or hypertension and progress to ESRD over 4–10 years. Amyloid deposition is seen in the kidneys, characteristically localised to the glomeruli, the spleen and rarely the liver, but is usually asymptomatic in the latter two sites.

Apolipoprotein A2 Amyloidosis (AApoA2)

The few kindreds described have slowly progressive proteinuric renal failure.

Gelsolin Amyloidosis (AGel)

This usually presents with corneal lattice dystrophy and progressive cranial neuropathy. Renal amyloid deposits are often subclinical but can occasionally cause ESRD.

Transthyretin Amyloidosis (ATTR)

In addition to neuropathy and cardiac involvement, up to a third of cases have evidence of proteinuria and renal failure and 10 % eventually develop ESRD. Gradually progressive autonomic neuropathy typically causes impaired bladder emptying requiring indwelling urinary catheters.

Investigations

Diagnosis of amyloidosis relies on a high index of clinical suspicion. Unfortunately amyloid is frequently asymptomatic until a relatively late stage and can then present with highly variable or nonspecific symptoms. Amyloidosis should be suspected in any patient with (a) nondiabetic nephrotic syndrome; (b) non-ischemic cardiomyopathy, particularly if the echocardiogram suggests concentric hypertrophy; (c) increased NT-proBNP in the absence of primary heart or renal disease;(d) hepatomegaly or increased alkaline phosphatase without an imaging abnormality; (e) peripheral and/or autonomic neuropathy; (f) unexplained facial or neck purpura; and (g) macroglossia. Any patient with suggestive features should undergo a biopsy to look for presence of amyloid deposits. Identification of amyloid should prompt a series of investigations to determine the amyloid fibril protein and organ involvement/dysfunction (Table 29.3).

Histology

The diagnosis of amyloidosis requires histological confirmation (Fig. 29.4). Amyloid deposits may be identified from a biopsy of a malfunctioning organ (e.g. kidney, heart or nerve in patient with nephrotic syndrome, cardiomyopathy or peripheral neuropathy, respectively) or via a screening biopsy when amyloid is suspected on clinical grounds. Subcutaneous fat biopsy, screening rectal biopsy and labial

Table 29.3 Investigation and staging of patient discovered to have amyloid deposits

Purpose and method		Note
Determining the amyloid type (*i.e. amyloid fibril protein*)		
Clinical presentation/features		Soft tissue amyloid (macroglossia/periorbital bruising/jaw claudication) – strongly suggestive of AL
		Amyloid cardiomyopathy – likely AL/ATTR
		Amyloid neuropathy – likely AL/ATTR
		Family history of amyloid – likely hereditary amyloidosis
Biochemical evaluation		Evidence of clonal dyscrasia (BJP, abnormal sFLC ratio, pp) – suggestive (but not diagnostic) of AL
		Evidence of chronic acute-phase response – suggestive (but not diagnostic) of AA
Immunohistochemistry		AA amyloidosis can be reliably excluded by negative immunohistochemical staining
		Sensitivity in AL and hereditary amyloidosis 70–90 % (i.e. frequent false-negative staining)
Mass spectrometry		Currently research technique. Likely gold standard in the future
Genetic sequencing		Frequently required when immunohistochemistry +/− mass spectrometry non-diagnostic of amyloid type
Determining amyloidotic organ involvement		
Clinical history and examination		Examine for macroglossia, carpal tunnel syndrome, postural hypotension, ecchymoses, ECOG performance status, 6-min walk test
SAP scintigraphy		To determine visceral organ involvement and whole-body amyloid load; serial scanning for monitoring
Cardiac evaluation		Echocardiography/Cardiac MRI/Tc-DPD scintigraphy/NT-proBNP/troponin T
Other organs		Quantification of proteinuria, renal function (GFR), liver function tests, tests of autonomic function
Characterising the underlying disease		
AL	Bone marrow biopsy	Include cytogenetic and flow cytometric analysis
	Serum immunoelectrophoresis	Monitor paraprotein throughout disease course
	Urine immunoelectrophoresis	Quantification of 24 h urine BJP, monitor BJP quantity throughout disease course
	Serum-free light chain assay (sFLC)	sFLC should be monitored during therapy and throughout disease course
	Skeletal survey	Look for lytic lesions
	Lymph node biopsy	Where indicated (absence of plasma cell dyscrasia, suggestion of lymphoma, IgM paraprotein)
	CT scanning of chest, abdomen, pelvis	Where indicated (absence of plasma cell dyscrasia, suggestion of lymphoma, IgM paraprotein)
	PET scanning	Where indicated (absence of plasma cell dyscrasia, suggestion of lymphoma, IgM paraprotein)
AA	Clinical syndrome	Rheumatoid arthritis, juvenile inflammatory arthritis, chronic infection, hereditary periodic fever
	Serological assays	Autoantibodies, CRP, SAA – SAA should be serially monitored throughout disease course
	Genetic sequencing	Sequencing of periodic fever genes (*MEFV*, *TNFRSF1A*, *MVK*)

BJP Bence Jones protein, *sFLC* serum-free light chain, *pp* paraprotein, *SAA* serum amyloid A protein, *CRP* C-reactive protein, *MRI* magnetic resonance imaging, *MEFV* familial Mediterranean fever gene, *TNFRSF1A* TRAPS gene, *MVK* mevalonate kinase gene

salivary gland biopsy are between 60 and 80 % sensitive. There have been concerns that organ biopsies in patients with amyloidosis carry an increased risk of haemorrhage, although firm evidence of this is lacking [19]. Congo red staining of amyloid produces pathognomonic apple green birefringence when viewed under cross-polarised light, and negatively stained electron microscopy reveals 8–15 nm diameter rigid, non-branching fibrils composed of twisted protofibrils of indeterminate length.

The main protein constituting the amyloid deposit can often be identified by immunohistochemistry, although this may be unreliable in AL and hereditary amyloidosis. Mass spectrometry can confirm the amyloid protein composition, and although this is currently a research technique, it will likely become the gold standard for identifying the amyloid fibril protein.

Imaging Amyloid Deposits

SAP Scintigraphy

SAP concentrates specifically in amyloid deposits of all types. Radiolabelled SAP scintigraphy has been used since 1988 in the UK for diagnosis and quantitative monitoring of amyloid deposits. This safe, noninvasive method provides information on the presence, distribution and extent of visceral amyloid deposits, and serial scans monitor progress and response to therapy. Unfortunately the method is not informative about amyloid deposition in the moving heart and is not commercially available.

Imaging the Heart

The classical two-dimensional Doppler echocardiographic appearance of cardiac amyloidosis is of concentric

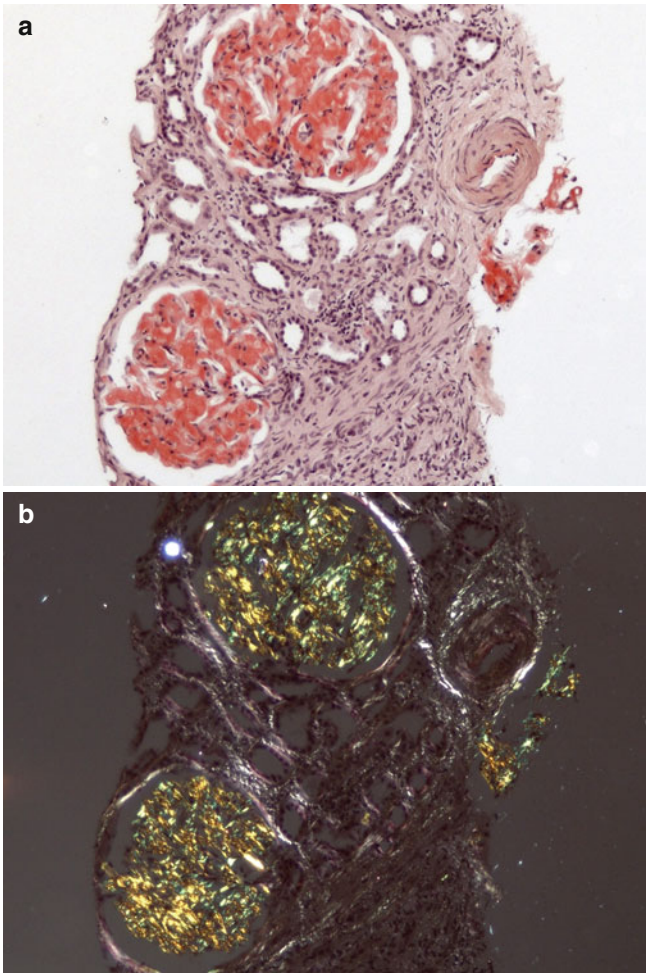

Fig. 29.4 Sections of renal biopsy stained with Congo red viewed under ×10 magnification. (**a**) Amorphous deposits of eosinophilic material are seen within the glomeruli. (**b**) Pathognomonic apple green birefringence of amyloid deposits when viewed under cross-polarised light

biventricular wall thickening with a restrictive filling pattern. Amyloid causes diastolic dysfunction with well-preserved contractility until a very late stage. The ECG in advanced disease may show small voltages and pathological 'Q' waves (pseudo-infarct pattern). The finding of abnormal gadolinium kinetics particularly global late gadolinium enhancement on cardiac magnetic resonance imaging has a high sensitivity and specificity for cardiac amyloidosis and has substantially contributed to diagnosis. Scans following injection of technetium-99m-labelled 3,3-diphosphono-1,2 propanodicarboxylic acid (^{99}Tc-DPD), an established bone tracer, are sensitive for detecting presence of cardiac ATTR amyloid deposits.

DNA Analysis

Hereditary amyloidoses are rare and often overlooked. Although all types are dominantly inherited, penetrance and expressivity are highly variable and there is frequently no obvious family history. DNA analysis is mandatory in all patients with systemic amyloidosis whose fibril type cannot be confirmed by immunohistochemistry or mass spectroscopy. Mutations encoding a number of amyloidogenic protein variants are known to cause hereditary amyloidosis, and both new variants and new amyloidogenic proteins are periodically identified [20].

Investigation of the Underlying Disease

AL Amyloidosis

All patients with AL amyloidosis should have the source of their amyloidogenic monoclonal light chain production investigated in detail. This should include a bone marrow examination, skeletal survey, serum and urine electrophoresis and immunofixation and serum FLC assay (Table 29.3).

AA Amyloidosis

An attempt to characterise the underlying inflammatory disease should be made in all cases of AA amyloidosis, although may be very difficult due to the diverse conditions involved (Tables 29.2 and 29.3). The precise cause of excessive SAA production remains undetermined in up to 10 % of patients with AA amyloidosis.

Treatment and Outcome

Principles of Treatment

Therapies aimed at enhancing amyloid clearance are under development, but at present the treatment of all types of amyloid centres on slowing new amyloid formation by reducing the supply of the amyloid fibril precursor protein and supporting or replacing compromised organ function. Treatment therefore requires precise identification of the amyloid fibril type. Successful inhibition of amyloid formation can result in net amyloid regression. Early diagnosis is the key to effective therapy.

Systemic AL Amyloidosis

The immediate goals of therapy are to rapidly eliminate production of misfolded amyloidogenic LCs with chemotherapy whilst minimising treatment toxicity and supporting target organ function. Effective management of AL amyloidosis requires a multidisciplinary approach. Consensus criteria for hematologic and organ responses were updated at the 12th International Symposium on Amyloidosis [21]. Achieving a hematologic response

translates into improved overall survival. Although partial responses can be beneficial, complete clonal responses are associated with the best clinical outcomes. A new paradigm for the treatment of AL amyloidosis has been proposed [22] in which both the underlying hematologic disorder and the end organ damage can be monitored with FLC and cardiac biomarkers to optimise therapy and minimise toxicity.

Treatment regimens, generally administered by haematologists, have been adapted from those developed in multiple myeloma, although most patients with AL amyloidosis have a low-grade plasma cell dyscrasia and small clonal burden. Treatment for AL amyloidosis is highly individualised and is based on age, cardiac staging and regimen toxicities (recently reviewed in [23]). Outcomes in AL amyloidosis have improved following introduction of effective chemotherapy regimens. Among 600 consecutive patients with AL amyloidosis evaluated in the UK, between 1990 and 2001, the median survival increased from 1.9 years for the cohort diagnosed between 1990 and 1995 to 3.3 years for the 1996–2001 cohort.

Response to Therapy in Patients with Renal Involvement

Close communication between the treating haematologist and nephrologist is crucial during chemotherapy for renal AL amyloidosis. Median survival in patients presenting with renal disease is 26.8–35.2 months in two studies including a total of 1,068 patients [14, 15]. Survival is strongly influenced by the degree of haematological response and the presence of cardiac amyloidosis, but not by the degree of renal dysfunction at presentation. More than 40 % of patients eventually received dialysis, and 13–26 % of cases presenting with potentially salvageable renal function (variously defined by baseline clearance of >20 mL/min or baseline creatinine of <5 mg/dL) progress to ESRD within a median of 12 months. Renal function deteriorated in almost 55 % within a median of 24 months in one study; conversely renal function improves in approximately a third of cases. CKD stage at baseline does not significantly influence renal response, whereas a more than 90 % FLC response to chemotherapy is associated with an almost fourfold increase in renal response and a 68 % reduction in the risk of renal progression. In a study of patients who had received stem cell transplantation, a renal response was seen in 71 % of patients who achieved a complete haematological response [24]. High-dose melphalan and stem cell rescue were associated with renal toxicity with an acute doubling of serum creatinine among 23 % of recipients but persistent renal decline in only 20 %. The potential nephrotoxicity of lenalidomide has been recently reported and demands careful follow-up of renal function.

Reactive Systemic, AA, Amyloidosis

In AA amyloidosis the aim of treatment is complete biochemical control of the underlying inflammatory disease, often carried out by the treating rheumatologist. The choice of therapy depends on the underlying disease process, but therapeutic success must always be assessed by measurement of the acute-phase response, ideally by serial SAA monitoring but or otherwise by monitoring CRP. Most patients with inflammatory arthritis have previously failed to respond to conventional disease-modifying antirheumatic drugs and many do well with anti-TNF therapies or other biologics such as anti-CD20 antibodies or anti-IL-1 or IL-6 therapies. In patients who fail to respond to these agents, there may still be a role for therapy with alkylating agents such as chlorambucil or cyclophosphamide. A multidisciplinary approach involving the nephrologist and, most frequently rheumatologist, is beneficial.

Median SAA concentration has been shown to be a strong predictor of both survival and renal outcome; persistent complete suppression of inflammation with normal SAA levels is associated with an almost 18-fold lower risk of death than median SAA levels of >155 mg/L. Median survival of 79–137 months has been recently reported in large series from Italy [25] and the UK. Approximately 40 % of patients will eventually require renal replacement therapy with a median time to dialysis from diagnosis of 78 months.

Dialysis-Related Amyloidosis (DRA)

The only effective treatment for DRA is successful renal transplantation, although drugs targeting the amyloid deposits are being tested. Serum levels of β_2-microglobulin fall rapidly following transplantation, and this is usually accompanied by an improvement in symptoms. This rapid response is probably due more to the anti-inflammatory properties of transplant immunosuppression and to discontinuation of dialysis than actual regression of deposits. In contrast to symptoms, radiological bone cysts heal slowly, and amyloid can be demonstrated histologically many years after renal transplantation. Attempts have been made to reduce DRA by altering the dialysis prescription. There is evidence that the risks of DRA are increased in patients dialysed using less 'biocompatible' membranes and that use of the more permeable membrane systems is relatively protective [26]. Greater removal of β_2-microglobulin is attained in patients undergoing high-flux haemodiafiltration and in the long-term these patients may be less prone to DRA [27]. The incidence of DRA appears to be falling, possibly reflecting the increasingly widespread use of such membranes [12]. Surgery may be required to relieve carpal tunnel compression, to stabilise the cervical spine or to treat bone fractures.

Hereditary Non-neuropathic Systemic Amyloidosis

These diseases, particularly lysozyme and apolipoprotein A-I amyloidosis, tend to run very indolent courses, and when renal failure is reached, transplantation can be successful with grafts surviving for decades. The rate of renal deterioration seems to be faster in fibrinogen amyloidosis, and the limited experience of renal transplantation suggests that amyloid deposition will cause graft loss after a median of ~7 years. As fibrinogen is synthesised solely in the liver, combined hepatorenal transplantation offers the possibility of 'surgical gene therapy' and complete protection from recurrent amyloidosis. The limitation of this approach is the serious risks associated with combined transplantation.

Preservation and Replacement of Organ Function

Organs infiltrated by amyloid may fail acutely often without obvious provocation. Attention must be paid to salt and water balance, maintenance of the circulating volume and prompt treatment of sepsis to reduce the risk of acute organ failure. Potentially nephrotoxic drugs, elective surgery and general anaesthesia are best avoided unless there are compelling indications.

Significant renal disease is present at diagnosis in at least 75 % of patients with systemic amyloidosis [28]. Nephrotic syndrome generally requires treatment with high-dose loop diuretics, and resistant cases may require addition of thiazide and/or potassium-sparing diuretics. Salt and, in many cases, fluid restriction may be advisable. In patients who have difficulty maintaining their intravascular volume, infusions of salt-poor human albumin can be very helpful.

Caution is required in the use of standard heart failure medications in patients with amyloidosis. Digoxin and calcium channel blockers have been associated with excess toxicity. Angiotensin-converting enzyme inhibitors can promote hypotension and should generally be avoided. Prophylactic amiodarone has been incorporated into therapy trials of amyloidosis to reduce the risk of sudden cardiac death if complex ventricular arrhythmias are detected on Holter ECG. The use of beta blockers in patients with cardiac amyloid may be associated with increased mortality. Diuretics are the mainstay of therapy but should be used with caution as amyloidosis causes a restrictive cardiomyopathy and high filling pressures are required to maintain cardiac output. Alpha agonists such as midodrine can improve orthostatic hypotension. Implantable cardiac defibrillators have been used, but their efficacy in this disease remains controversial.

In highly selected younger patients with isolated irreversible cardiac failure, heart transplantation offers a possibility of long-term survival and has been performed in a small number of patients. The scarcity of donor hearts, the high transplant-related mortality and the risk of amyloid deposition in the graft make rigorous patient selection mandatory. In AL amyloidosis chemotherapy is required after cardiac transplantation to prevent graft amyloid or its progression in other organ systems.

Renal Dialysis

The outcome of AL amyloidosis patients on long-term dialysis is improving, but survival is reduced compared to age-matched nondiabetic patients with other diseases [14, 15]. Patients who commenced dialysis after 2002 in the UK had a median survival of 43.6 months, whereas data from the USA and Italy report median survival of 10.4–11 months. The outcome in patients with other types of amyloid is more favourable [25, 29]. In AA amyloidosis median survival on dialysis has been reported between 17 months in earlier series and 69 months, the latter in a cohort of 129 patients with an incident mortality of 18 % and less than 10 % mortality in subsequent years [30].

Renal Transplantation

Although early mortality is increased, due to sepsis and cardiac failure, long-term renal graft survival and rejection rates are comparable with other systemic diseases [31]. Less than 10 % of patients who reached ESRD due to AL amyloidosis receive a renal transplant; median patient and graft survival in these highly selected patients were 89 months [15]. In a few cases renal transplantation has been followed by autologous stem cell transplantation with stable renal function in 4/8 patients [32]. Recent experience of renal transplantation in selected patients with AA amyloidosis has shown 5- and 10-year graft survival of 74 and 68 %, respectively. These encouraging data have prompted use of living donor renal transplants. Most patients have a functioning graft until death [15], despite frequent histological presence of amyloid deposits in the renal allograft.

Novel Therapies and Perspectives

The remarkable advances in the understanding of the molecular mechanisms involved in amyloid formation and tissue damage achieved in the last decade have revealed several new drug targets (Fig. 29.1). Several new approaches have been developed:

- Synthesis of the amyloidogenic precursor may be silenced by RNA interference or by antisense oligonucleotides.

- Inhibitors of proteases (secretase) are being evaluated in trials.
- Inhibitors of glycosaminoglycans binding to the amyloid proteins (eprodisate) are being evaluated for treatment of secondary amyloidosis in clinical trials.
- Small molecules capable of stabilising the amyloid precursor and preventing its misfolding and aggregation (diflunisal, tafamidis) are being tested in ATTR amyloidosis.
- SAP can be cleared from amyloid deposits by using small palindromic drugs (e.g. CPHPC).
- The clearance of amyloid deposits can be promoted and accelerated by specific antibodies through passive and active immunotherapy or by combining CPHPC with anti-SAP antibodies.
- Small molecules, such as iododoxorubicin and doxycycline, are able to disrupt the amyloid fibrils and reduce amyloid burden.

Clinicians should be aware that in the near future, amyloid diseases will be treated with combination approaches that reduce protein precursor production, prevent aggregation, and induce fibril resorption.

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