

The Prevalence and Management of Systemic Amyloidosis in Western Countries

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Key Words

Diagnosis · Monitoring · Precursor proteins · Treatment · Typing

Abstract

Background: Amyloidosis has been a mystery for centuries, but research of the last decennia has clarified many of the secrets of this group of diseases. A protein-based classification of amyloidosis helps to understand problems that were part of the obsolete clinical classification in primary, secondary, and familial amyloidosis. All types of amyloid are secondary to some underlying precursor-producing process: each type is caused by a misfolded soluble precursor protein that becomes deposited as insoluble amyloid fibrils. **Summary:** The incidence of amyloidosis is not well documented, but probably falls between 5 and 13 per million per year. Prevalence data are scarce, but one UK study indicates about 20 per million inhabitants. Amyloidosis can be localized (amyloid deposited in the organ or tissue of precursor production) or systemic (amyloid at one or more sites distant from the site of precursor production). The major systemic types of amyloidosis are AL (associated with a light chain-producing plasma cell dyscrasia), AA (associated with longstanding inflammation), wild-type ATTR (associated with normal

transthyretin and old age), and hereditary ATTR (associated with a transthyretin mutation) amyloidosis. Imaging techniques, such as cardiac ultrasound, magnetic resonance imaging, bone scintigraphy, and serum amyloid P component scintigraphy, are useful both for diagnosing amyloidosis and for assessing disease severity. Serologic markers are useful for detecting organ disease and disease monitoring during follow-up. Current treatment modalities are directed against the ongoing supply of precursor proteins and thereby aim to stop further accumulation of amyloid. Novel treatment modalities, such as interference with amyloid formation and even removal of amyloid, are being studied. A well-thought and planned monitoring during follow-up helps to assess the effect of treatment and to early detect possible progression of amyloidosis. **Key Messages:** Clinical management comprises histologic proof of amyloid, evidence of systemic deposition, reliable typing, precursor assessment, severity of organ disease, risk assessment and prognosis, choice of treatment, and planned monitoring during follow-up. **Facts from East and West:** (1) AL amyloidosis is the most prevalent

For the clinical presentation and management of systemic light-chain amyloidosis in China, see Huang and Liu, *Kidney Dis* 2016;2:1–9.

type of amyloidosis accounting for 65% of the amyloidosis-diagnosed patients in the UK and for 93% of the amyloidosis-diagnosed patients in China. The predisposition of men over women to develop AL amyloidosis might be higher in China than in Western countries (2:1 vs. 1.3:1). Both in the East and West, incidence increases with age. At the time of diagnosis, edema is twice as frequent and the proportion of renal involvement is higher in Chinese compared to Western patients. (2) Melphalan followed by autologous stem cell transplantation (ASCT) is the current standard therapy but is restricted to eligible patients. The efficacy and safety of bortezomib combined with dexamethasone were proven in Western patients and recently confirmed in a Chinese cohort. Recent studies in China and the US indicate that bortezomib induction prior to ASCT increases the response rate. Thalidomide and lenalidomide have shown benefit, but toxicity and lack of clinical evidence exclude these agents from first-line therapy. The green tea extract epigallocatechin-3-gallate is under investigation as an inhibitor of AL amyloid formation and a compound that might dissolve amyloid.

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Introduction

History

Probably the first description of a patient with amyloidosis was an autopsy case in 1639, published by Nicolaes Fonteyn, a physician and well-known Dutch poet of his time, who lived in Amsterdam. In the following centuries, many other such autopsy cases have been described. The name amyloid for this amorphous and hyaline change in tissue has been derived from the iodine-staining reaction of this material that resembles that of starch (the Greek word for starch is amylo). Rudolph Virchow, a famous German pathologist, used the term amyloid for the first time in 1854. The Congo red stain was introduced in 1922 by Bennhold to identify amyloid deposits in tissue. In 1927, two Belgian researchers, Divry and Florkin, described the characteristic apple-green birefringence of Congo red in polarized light. The fibrillary nature of amyloid was detected in 1959 by the Americans Cohen and Calkins when the material was viewed under the electron microscope [1].

Classification

Because of the uniform staining characteristics of amyloid, clinicians were puzzled by the variety of different diseases that were associated with amyloid deposition. The recognition of many inflammatory underlying dis-

eases led to the notion of so-called secondary amyloidosis. On the other hand, diseases such as multiple myeloma and monoclonal proteins were also known to be associated with amyloid. Occasionally, there was a clear familial pattern of inheritance, but frequently the disease seemed to appear suddenly and was therefore thought to be a primary form of amyloidosis. Some pathologists tried to characterize the type of amyloid because of a particular perireticular or pericollagenous histological picture, but eventually this approach was not really useful. So, for a long time, amyloidosis was classified clinically in primary and secondary types, in familial and localized types, and in other types. This clinical classification was useful but not fully satisfactory.

In 1969, a fundamental breakthrough came because of a finding of the Israeli researcher Mordechai Pras. He discovered that, although amyloid itself was thought to be insoluble, the characterizing protein could be solubilized from amyloid in distilled water [2]. This enabled him, and many researchers after him, to chemically characterize the protein of each particular type of amyloid. In the years thereafter, many chemically different types of amyloid were discovered, leading to the current useful protein-based classification of amyloidosis [3].

From a clinical viewpoint, it is still important to first investigate whether or not amyloid deposition is limited to the organ or tissue where its precursor protein is produced. If so, amyloid deposition is localized. Amyloid is deemed to be systemic if the precursor protein has been produced in a different part of the body, such as bone marrow or liver, and transported by the blood stream to a distant site of amyloid deposition [3].

The three major types of systemic amyloidosis are AL, AA, and ATTR (transthyretin-derived) amyloidosis. AL amyloidosis is caused by deposition of soluble immunoglobulin λ or κ free light chains (FLCs) as insoluble amyloid fibrils. Usually, a low-grade plasma cell clone in the bone marrow is responsible for the supply of the involved FLC. AA amyloidosis is caused by deposition of serum amyloid A protein (SAA), an acute phase protein. Usually, an underlying chronic inflammatory disease is responsible for chronically increased levels of soluble SAA that becomes misfolded and deposited as insoluble amyloid fibrils. ATTR amyloidosis is caused by deposition of transthyretin (TTR). Two types can be distinguished: a hereditary type and a type associated with old age. Mutated TTR plays a role in the hereditary type, whereas normal (wild-type) TTR plays a role in the type seen in old age. In both types, soluble TTR becomes misfolded and deposited as insoluble amyloid fibrils [3].

Incidence and Prevalence

Incidence

Few data are available to obtain a reliable estimate of the current incidence and prevalence of systemic amyloidosis. An older small American study of 1992 about the incidence of AL amyloidosis in Olmsted county in Minnesota is still useful. In this study, the overall age- and sex-adjusted 95% confidence interval was 5.1–12.8 per million inhabitants per year in the study period from 1950 to 1989 [4]. Recently, in a 2013 study in the UK, the estimated incidence of all systemic amyloidosis exceeds 8.0 per million inhabitants per year (AL 65%, AA 18%, wild-type ATTR 7%, and hereditary ATTR 10%) [5]. In a 2012 study from Sweden using data of 949 patients from 2001 to 2008, the estimated incidence of nonhereditary amyloidosis was 8.3 per million inhabitants per year: 3.2 for AL amyloidosis and 2.0 for AA amyloidosis. The diagnostic age with the highest incidence was over 65 years [6]. In a 2013 Swedish study, 221 patients with hereditary ATTR amyloidosis were found, resulting in an incidence of 2.0 per million per year. However, Sweden is one of the few countries with a large focus of hereditary ATTR amyloidosis. The highest incidence in an affected area was 100 times higher than in the rest of Sweden [7]. The risk of developing AA amyloidosis may be higher in countries with endemic infectious diseases (e.g. tuberculosis or leprosy) and autoinflammatory diseases (e.g. familial Mediterranean fever in the countries around the Mediterranean sea).

Prevalence and Survival

Very little data are available about the prevalence of systemic amyloidosis. The estimated minimum prevalence of systemic amyloidosis in the UK is 20 per million inhabitants [5]. The prevalence of AA amyloidosis is higher than that of AL amyloidosis in developing countries because of a higher prevalence of associated underlying infectious diseases. Wild-type ATTR amyloidosis affects 25% of the very old people (over 85 years of age) in Finland [8]. About 13% of the patients above 60 years having heart failure with preserved ejection fraction were thought to suffer from wild-type ATTR amyloidosis in a Spanish study [9]. In a recent British study on wild-type ATTR amyloidosis, 8 times more men than women were affected [10]. In most studies of patients with AL amyloidosis, the proportion of men is somewhat higher (about 1.1–1.3) than that of women. The reverse (more women than men) is observed in AA amyloidosis, because of a high proportion of patients (mainly women) with underlying rheumatoid arthritis [11].

The prognosis of untreated patients was documented in older studies: the median survival was about 6–12 months in AL amyloidosis and about 3–4 years in AA amyloidosis [11]. The median survival of untreated patients with hereditary ATTR amyloidosis is almost 10 years, although some patients may survive up to 15 years. The median survival of wild-type ATTR amyloidosis is about 5 years. Using rather broad data of the Swedish study, the estimated median survival was 3 years for AL amyloidosis, 4 years for AA amyloidosis, and 6 years for localized amyloidosis [6]. The median survival for all systemic amyloidoses in the UK is about 32 months [5].

Disease Management: Stepwise Approach

When a clinician meets a patient suspected to have amyloidosis, a stepwise approach is advised. The first step is to confirm the diagnosis using a tissue biopsy. The second step is searching for convincing evidence of localized or systemic amyloid deposition. The third step is unequivocal typing of amyloid, followed by detection and/or quantification of the serum precursor as the fourth step. The fifth step is a thoughtful clinical assessment of severity of organ disease, associated risks, and prognosis. The sixth step is a balanced choice of the most effective treatment with acceptable risks. The final, seventh, step is planned monitoring of the course of the disease and the effect of treatment during follow-up [12].

Diagnosis

Awareness

Awareness is essential for timely detection and treatment of amyloidosis. A clinical suspicion of amyloidosis may rise in finding otherwise unexplained phenomena, such as organomegaly (tongue, liver, or spleen), proteinuria, right-sided cardiac failure, orthostatic hypotension, peripheral polyneuropathy, autonomic neuropathy, and malabsorption. This is even more compelling if more than one of these phenomena are present. But also a family history of hereditary amyloidosis, proteinuria appearing in a patient with longstanding inflammation, unexpected symptoms in a patient with multiple myeloma or monoclonal gammopathy of undetermined significance, and an elderly man with heart failure and biventricular hypertrophic cardiac walls all may indicate the presence of systemic amyloidosis.

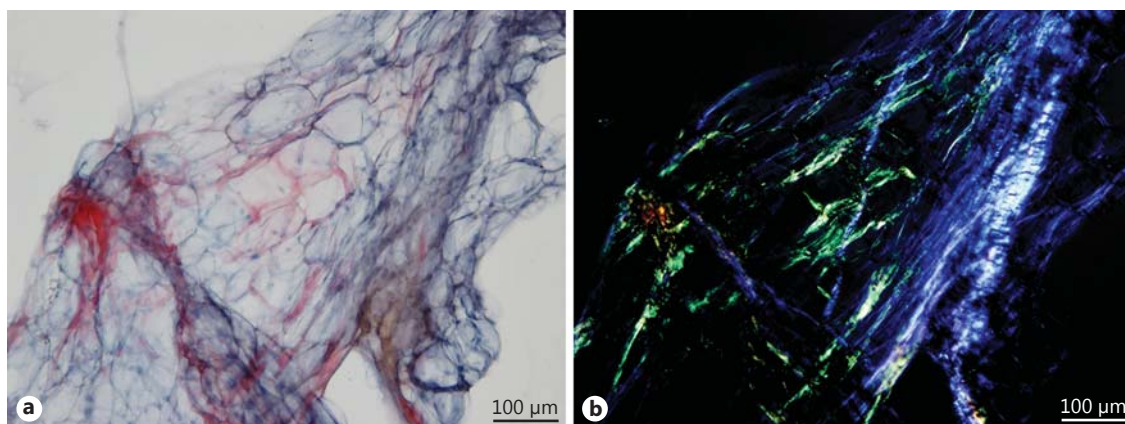


Fig. 1. A subcutaneous abdominal fat specimen containing amyloid deposits, stained with Congo red. **a** Amyloid deposits are stained red when observed in bright light. **b** Amyloid deposits show green birefringence when observed in polarized light (collagen is bluish-grey). Scale bar = 100 μm .

Detection

Amyloid can be detected by a screening biopsy in a different site of the body than where the symptoms are. After centuries of only autopsy studies, the perspective turned in 1960 when the Israeli clinicians Gafni and Sohar showed the utility of a rectal biopsy to detect amyloid in alive patients [13]. This screening method was soon generally adopted and remained for many years the gold standard for screening. Ten years later, the Swedish pathologists Westermark and Stenkvist introduced the subcutaneous fat tissue aspiration as an elegant and even less invasive screening method [14]. This method became popular in the US and in some European countries such as the Netherlands and Germany [15]. In other European countries, such as Spain and France, the labial salivary gland biopsy was preferred [16].

Awareness, however, is frequently absent because of the rarity of amyloidosis. In that case, the diagnosis is often made by the pathologist, confronted with unexpected amorphous eosinophil tissue deposits. After staining with Congo red dye, the red-stained amyloid deposits turn to green in polarized light (fig. 1), confirming the diagnosis. The Congo red stain is undisputed the gold standard for amyloid detection [3]. Electron microscopy can help to show typical amyloid fibrils in some ambiguous cases in which the Congo red stain fails to yield clear results. Recently, Swedish investigators introduced new staining methods using fluorescent oligothiophenes (e.g. hFTAA) that may have a higher sensitivity and may make it easier to detect amyloid, but probably at the expense of a somewhat reduced specificity [17].

Typing

Immunohistochemistry is the preferred method for routine typing of amyloid in tissue, using specific antibodies. In AA amyloidosis, this technique is sufficient, provided that sensitive and specific monoclonal antibodies are used. However, immunohistochemistry is less reliable in ATTR amyloidosis and frequently even useless in demonstrating AL amyloidosis [18, 19]. DNA analysis of the TTR gene may help to demonstrate a particular TTR mutation. And an increased level of one of the two immunoglobulin light chains may be a strong indicator of AL amyloidosis. However, unequivocal typing is extremely relevant because treatment differs considerably among the different types of amyloidosis. Therefore, in cases without convincing evidence obtained by immunohistochemistry and/or with a clinical picture that can be part of AL as well as ATTR amyloidosis (e.g. isolated cardiomyopathy in an elderly man) or AL as well as many other rare genetic types (e.g. nephrotic syndrome in an atypical patient), proteomics is the preferred method to type the amyloid involved.

Proteomics has recently been developed to characterize the protein content of the amyloid by techniques such as mass spectrometry after isolating the amyloid deposits by laser microdissection [20]. These proteomics techniques have been applied successfully to both tissue biopsies and abdominal fat tissue aspirates by the American Vrana and the Italian Lavatelli [20, 21]. Therefore, difficult cases should be typed with confidence by this method. But it is still too early to replace immunohistochemistry as a routine method by these proteomic tech-

niques, because they are very expensive, time-consuming, and only available in some highly specialized centers.

Disease Manifestations and Severity

Organ Disease and Amyloid Type

AA amyloidosis is usually caused by some underlying inflammatory process. The usual presentation is kidney disease as reflected by proteinuria or loss of renal function. Proteinuria may vary from only a positive dipstick to frank nephrotic syndrome with massive edema. Autonomic dysfunction may cause abdominal complaints such as diarrhea and disturbed gastric emptying. Hepatomegaly and cardiomyopathy are seen, although infrequently.

AL amyloidosis is usually caused by an elevated λ or κ FLC, produced by an underlying plasma cell dyscrasia. The presentation is diverse and can vary from vague symptoms such as weight loss or fatigue to severe nephrotic syndrome, right-sided heart failure, diarrhea, or liver failure. Edema can be a sign of nephrotic syndrome, of heart failure, of liver failure, or even of all together at the same time. Hypotension can be caused by heart failure, nephrotic syndrome, and/or autonomic dysfunction. An enlarged tongue with indentations (glossomegaly) and periorbital ecchymosis are signs almost pathognomonic of AL amyloidosis.

The hereditary type of ATTR amyloidosis is caused by one of more than hundred TTR mutations. The usual clinical presentations are peripheral axonal polyneuropathy, autonomic neuropathy, carpal tunnel syndrome, cardiomyopathy, and/or vitreous opacities. Loss of renal function can directly be caused by amyloid, but also indirectly because of neurogenic bladder caused by the neuropathy.

The acquired wild-type ATTR amyloidosis is seen at old age, and its cause is unknown. Although amyloid deposits can be found in women with this disease, the clinical picture of a slowly progressive cardiomyopathy is more frequently seen in men than in women. Carpal tunnel syndrome often precedes the clinical picture of cardiomyopathy by a couple of years.

A β 2M amyloidosis was, until some years ago, frequently observed in patients who underwent hemodialysis for more than 5 years. The precursor of this type of amyloid is β 2-microglobulin, a middle molecule that could not be removed effectively by the older dialysis membranes, such as cuprophan. The frequency of this

type of amyloidosis dropped after the introduction of high-performance dialysis techniques and novel dialysis membranes, such as polyacrylonitrile. The disease has become less frequent but did not disappear. The usual presentation is in the joints, beginning as shoulder pain and carpal tunnel syndrome, and progressing after some years by causing large periarticular cysts, fractures, and destructive spondyloarthropathy. Involvement of other organs, such as the heart, tongue, and large bowel, is rarely seen after many years [22].

AL, AA, and ATTR amyloidosis are by far the most frequent types of amyloidosis [12]. However, one should always keep in mind that a patient may suffer from one of the other eleven, even rarer systemic types of amyloidosis [3]. Keeping this in mind is particularly useful for nephrologists, because most of these rarer types are associated with renal disease, such as immunoglobulin heavy chain (AH), apolipoprotein AI (AApoAI), apolipoprotein AII (AApoAII), apolipoprotein AIV (AApoAIV), gelsolin (AGel), lysozyme (ALys), leukocyte chemotactic factor-2 (ALect2), and fibrinogen α (AFib) [3].

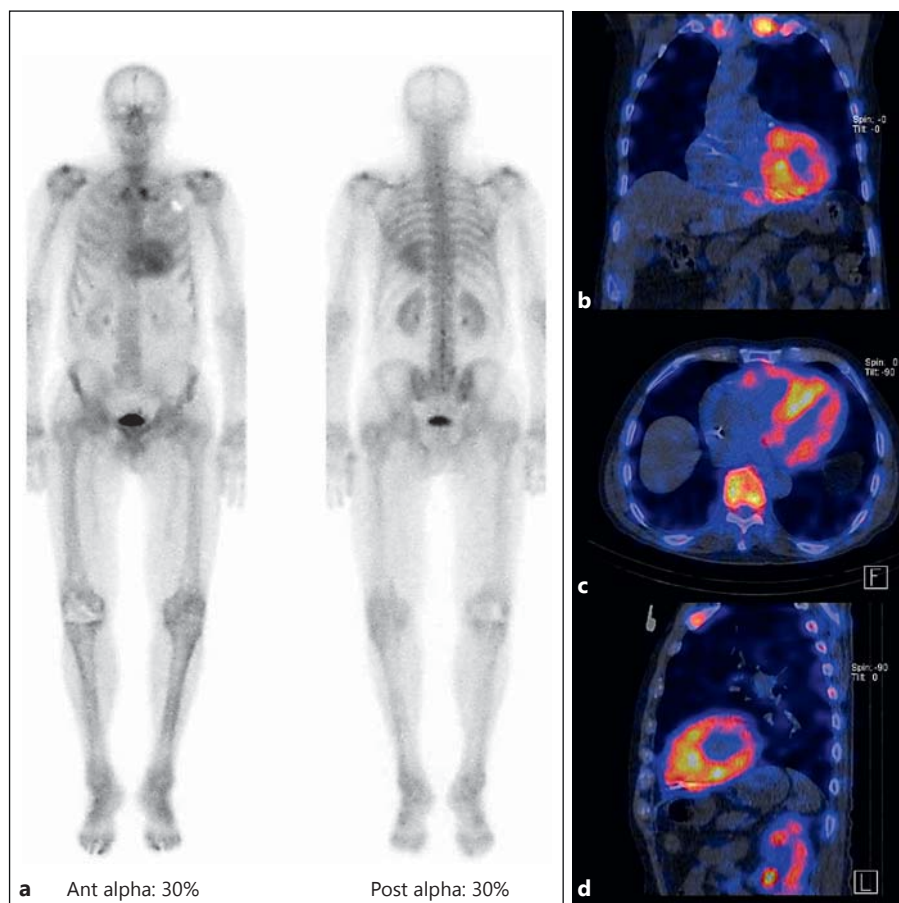
N-terminal pro-brain natriuretic peptide (NT-pro-BNP) and troponin T are sensitive indicators of heart involvement; serum creatinine, albumin, and proteinuria are sensitive markers of kidney involvement, and alkaline phosphatase (AP), γ -glutamyl transferase, albumin, and bilirubin are suitable markers of liver involvement [12].

Imaging

Plain radiographs and computed tomography (CT) scans are useful to detect pulmonary and pleural disease, restrictive cardiac disease (the combination of a normally sized heart and right-sided heart failure), and joint involvement (increased soft tissue of the shoulders, wrists, or hips). Abdominal ultrasound, CT, and magnetic resonance imaging (MRI) can detect organomegaly by assessing the sizes of visceral organs, such as the liver, spleen, and kidneys.

Cardiac ultrasound is the preferred method for detecting cardiac amyloidosis in general, whereas bone scintigraphy using pyrophosphate or diphosphonates can differentiate between the ATTR (positive uptake) and AL (minimal or no uptake) type of cardiac amyloidosis (fig. 2) [23]. Cardiac MRI, especially with late gadolinium enhancement, has a superior diagnostic value in case of unequivocal cardiac ultrasound results [24]. T1 myomapping is a new and promising MRI technique developed in the UK by Moon and colleagues [25] for detection and in particular for monitoring of cardiac amyloidosis. MIBG scintigraphy may help to detect early cardiac disease [26].

Fig. 2. Technetium (^{99m}Tc)-hydroxymethylene diphosphonate bone scintigraphy in a 71-year-old man with wild-type ATTR amyloidosis. **a** Total body scan with increased cardiac uptake and soft tissue uptake. The skeletal uptake is relatively diminished. Coronal (**b**), transverse (**c**), and sagittal (**d**) single photon emission computed tomography/CT images of the heart showing increased uptake in mainly the left but also the right ventricular wall. Ant = Anterior view; Post = posterior view.



FDG-PET scintigraphy frequently shows positive uptake in localized amyloidosis, whereas no uptake is found in systemic amyloidosis [27]. The German Kollmer showed recently that magnetic resonance neurography may detect structural nerve damage earlier and with a higher sensitivity than gold-standard nerve conduction studies in hereditary ATTR amyloidosis [28].

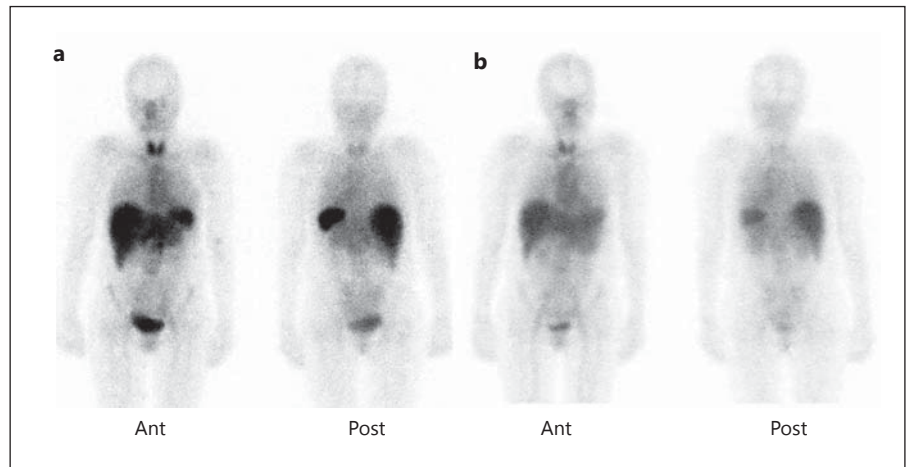
Serum amyloid P component (SAP) scintigraphy, developed in the UK by Pepys and Hawkins, is currently the best technique to image amyloid deposition in the spleen, liver, kidneys, adrenal glands, joints, and bone marrow, but unfortunately fails to image the heart. SAP is a pentraxin isolated and purified from blood of healthy donors that binds in a calcium-dependent way to all types of amyloid. In individual cases, it can be a useful technique to monitor the amyloid load of the body and of individual organs during the course of the disease and after treatment (fig. 3). Disadvantages are its lack of general availability and the inability to image the heart [29].

Organ Disease and Risk Assessment

Involvement of four organs should be investigated in all patients: the heart, kidney, liver, and peripheral nervous system. After the international amyloidosis symposium in France in 2004, criteria for organ involvement and treatment response in AL amyloidosis were defined in a consensus paper. The most relevant clinical data were proteinuria >0.5 g/day (for the kidney), mean cardiac wall thickness >12 mm (for the heart), liver span >15 cm or AP >1.5 times the upper limit (for the liver), and clinical data indicating peripheral or autonomic nerve disease. Criteria were also described for the involvement of the intestines, lungs, and soft tissues. In the same paper, response criteria were described for the heart, kidney, liver, and nerve [30]. The response criteria were recently updated by adding the FLC and the NT-proBNP responses [31].

Many publications have shown that especially cardiac involvement is a prognostic bad factor as well as involvement of ≥ 3 of the four major organs. Mayo Clinic inves-

Fig. 3. Total body ^{123}I -SAP scintigraphy (anterior and posterior images) of a 48-year-old woman with systemic AL amyloidosis. **a** At the start of treatment, scintigraphy shows increased uptake in the liver (++++) and spleen (+++). Nonspecific uptake can be seen in the thyroid gland, nasopharynx, and stomach. **b** Two years after successful chemotherapy resulting in complete response, the SAP scan shows clear improvement with some residual uptake in the liver (+) and spleen (+). Ant = Anterior view; Post = posterior view.



tigators showed that the difference between the involved and uninvolved FLC as well as cardiac markers such as NT-proBNP and troponin T can be used to stratify patients with AL amyloidosis into four risk categories [32].

Treatment

Precursor-Product Concept

Stopping the supply of a precursor by the elimination or suppression of an underlying precursor-producing process is the so-called ‘precursor-product’ concept. When the supply of necessary precursors disappears, it will halt ongoing deposition of amyloid. Using this concept, it is important to diagnose amyloidosis early and to start treatment as early as possible, thereby stabilizing the disease and preventing further progression.

AA amyloidosis should be treated by decreasing SAA serum levels to normal basal values (<3 mg/l). If this level can be reached and maintained below 10 mg/l, the 10-year survival rises to 90%, whereas in the group with median SAA levels above 10 mg/l the 10-year survival falls down below 40% [33]. The best way to achieve a normal basal serum value of SAA is by complete suppression or eradication of the underlying chronic inflammatory disease. This means eradication of the infection by antibiotic treatment sometimes combined with surgery in patients with infectious diseases such as tuberculosis, leprosy, recurrent pulmonary infections, and osteomyelitis. Chronic inflammatory diseases such as rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, and Crohn’s disease can nowadays be treated by effective anti-inflammatory drugs such as methotrexate and biologi-

cals, especially those directed against tumor necrosis factor (TNF) and interleukin-1 (IL-1). If these drugs can be used, effective suppression of SAA to low or even normal serum concentrations is often possible. Autoinflammatory diseases such as familial Mediterranean fever, TNF-receptor-associated periodic syndromes, the hyper-IgD syndrome, and cryopyrin-associated periodic syndromes often respond to some of these biologicals, especially to anakinra that is directed against IL-1 [34]. Tocilizumab, an anti-IL-6 receptor antibody, directly suppresses the production of C-reactive protein and SAA by the liver [35].

AL amyloidosis should be treated by eradicating the underlying plasma cell dyscrasia using chemotherapy, thereby decreasing the abnormally increased FLC blood level to the normal range [36]. High-dose melphalan followed by autologous stem cell transplantation (ASCT) in eligible patients has shown clear benefits [37]. In an American study, the low-risk group that was eligible for ASCT had a median survival of 4.6 years. One randomized clinical trial from France, however, seriously questioned the favorable results of high-dose melphalan followed by ASCT [38]. Meanwhile, many more studies of novel drugs, such as thalidomide, bortezomib, lenalidomide, pomalidomide, and MLN9708, have shown clear effects, often with the best effects in combination with dexamethasone [39]. Current treatment schedules stratify patients for different types of chemotherapy [32, 39]. The survival of responding patients still increases, but early deaths due to advanced cardiac dysfunction at presentation remain a big problem. The median survival of untreated patients with advanced cardiac involvement is 3–6 months and does not really change by any treatment.

The search for an effective and less dangerous treatment will go on.

Liver transplantation was the only available treatment in hereditary ATTR amyloidosis until recently, removing the source of 99% of the circulating mutated TTR. The median survival after liver transplantation indeed increased to more than 20 years, and the 10-year survival is about 85% [40]. However, this approach is not always successful, because ATTR amyloid sometimes progresses in the heart after liver transplantation. It looks as if wild-type ATTR amyloidosis takes over and further progresses in these transplanted patients. Because of this, patients with late disease onset (often males with cardiomyopathy) and patients with non-TTR-Met30 mutations are deemed less eligible for liver transplantation [40, 41].

Besides treating the underlying precursor-producing process, it is also important to provide supportive treatment for decreased organ function caused by amyloid deposition. Involvement of more than one vital organ frequently results in an intangible knot of serious problems such as edema, hypotension, dyspnea, cardiac failure, renal failure, fatigue, and loss of weight. Late in the disease process, it is often difficult and sometimes impossible to find an acceptable solution for all problems. In order to get the best results of all treatments for the individual patient with amyloidosis it is necessary that all medical specialists involved closely collaborate and that one of them coordinates the collective efforts.

Emerging Treatments

Novel treatment modalities are currently being developed and tested in clinical trials. Not only reduction of the precursor supply but also interference with the formation and deposition of amyloid and removal of amyloid deposits are treatment modalities being investigated.

Gene silencing is a novel and highly promising way to reduce the supply of precursor protein: selective interference with the process of gene transcription and translation to the TTR protein by antisense or small interfering RNA in the liver results in very low (about 10–20% of normal) TTR serum levels [42, 43]. This might also be a useful approach in AL amyloidosis by targeting the amyloidogenic light-chain messenger RNA, as has been shown to be effective in a plasmacytoma mouse model [44].

Interference with amyloid formation by stabilizing the precursor protein has been shown to be successful in ATTR amyloidosis: both diflunisal and tafamidis stabilize the TTR tetramer and thereby reduce the normal breakdown of tetramers into dimers and monomers that are

amyloidogenic. Two clinical trials showed a reduction in the progression of polyneuropathy in patients with hereditary ATTR amyloidosis [45, 46]. Also, in AA amyloidosis, interference with polymerization and deposition of amyloid fibrils in extracellular tissue by eprodisate (a glycosaminoglycan-mimetic drug) slowed the decline of renal function in a clinical trial [47]. The green tea extract epigallocatechin-3-gallate seems to have an inhibitory effect on the formation of AL and ATTR amyloid [48]. A German clinical trial has started to evaluate epigallocatechin-3-gallate in promoting the regression of residual cardiac damage in patients with AL amyloidosis who successfully completed chemotherapy.

The combination of doxycyclin and tauroursodeoxycholic acid was capable to stimulate the removal of ATTR amyloid deposits in mice. An Italian phase 2 study of this drug combination has been started in patients with ATTR amyloidosis [49]. This might also be a useful approach in AL amyloidosis by reducing fibril formation as shown in a transgenic mouse model [50].

A different approach is to remove amyloid using conformation-specific antibodies [51]. A phase 3 trial in AL amyloidosis will start soon. Another interesting development is the combined use of a drug called CPHPC and anti-SAP antibodies. CPHPC [the abbreviation of (R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid] is a drug that effectively depletes SAP from the circulation. This mechanism possibly stops accumulation of amyloid and may be useful for all types of systemic amyloidosis, although fibrinogen-derived amyloidosis seems to respond best of all. The Royal Free group in London recently demonstrated in mice with AA amyloidosis that CPHPC followed by anti-SAP antibodies resulted in a quick removal of almost all AA amyloid from the tissues [52]. A pilot study in 15 patients with different types of systemic amyloidosis safely triggered clearance of amyloid deposits from the liver and some other tissues [53].

Disease Monitoring

Disease monitoring is essential. Repeated measurements are useful to monitor the effect (or lack of effect) of treatment and detect progression as early as possible. Two different processes should be monitored.

The first is the underlying production of the precursor proteins SAA in AA amyloidosis and the involved FLC in AL amyloidosis. Successful treatment should bring the SAA levels down continuously below 3 mg/l, and the involved FLC levels and the κ/λ ratios should fall within the reference ranges.

The second is amyloid accumulation, the clinical ‘amyloid load’. Quantitative clinical measurements are available for monitoring this clinical ‘amyloid load’, such as the serum (and urine) markers NT-proBNP and troponin T (for the heart); serum creatinine, creatinine clearance, albumin, and proteinuria (for the kidney), and AP, γ -glutamyl transferase, albumin, and bilirubin (for the liver). Besides, there are other measurements, such as measurements of ventricular wall thickness, ejection fraction, conduction, and rhythm (for the heart); of heart rate variability, the Ewing battery, gastric emptying (autonomic function), and the sizes of enlarged organs, such as

the liver, spleen, and kidneys. Imaging techniques, such as SAP scintigraphy (fig. 3) for an overview and subcutaneous fat tissue samples (fig. 1) for an inside tissue view are also suitable for monitoring [12, 54].

Conflict of Interest Statement

H.L.A.N. and J.B. declare that they have no conflicts of interest. B.P.C.H. is chair of a data safety monitoring board of GSK, has done consultancy work for Pfizer, was a member of a clinical advisory board of Neurochem, and received honoraria or consultation fees from Pfizer, GSK, Neurochem, UCB, and Abbott.

References

- Kyle RA: Amyloidosis: a convoluted story. *Br J Haematol* 2001;114:529–538.
- Pras M, Zucker-Franklin D, Rimón A, Franklin EC: Physical, chemical, and ultrastructural studies of water-soluble human amyloid fibrils. Comparative analyses of nine amyloid preparations. *J Exp Med* 1969;130:777–796.
- Sipe JD, Benson MD, Buxbaum JN, Ikeda S, Merlini G, Saraiva MJ, Westermark P: Nomenclature 2014: amyloid fibril proteins and clinical classification of the amyloidosis. *Amyloid* 2014;21:221–224.
- Kyle RA, Linos A, Beard CM, Linke RP, Gertz MA, O’Fallon WM, Kurland LT: Incidence and natural history of primary systemic amyloidosis in Olmsted County, Minnesota, 1950 through 1989. *Blood* 1992;79:1817–1822.
- Pinney JH, Smith CJ, Taube JB, Lachmann HJ, Venner CP, Gibbs SD, Dungu J, Banyersad SM, Wechalekar AD, Whelan CJ, Hawkins PN, Gillmore JD: Systemic amyloidosis in England: an epidemiological study. *Br J Haematol* 2013;161:525–532.
- Hemminki K, Li X, Försti A, Sundquist J, Sundquist K: Incidence and survival in non-hereditary amyloidosis in Sweden. *BMC Public Health* 2012;12:974.
- Hemminki K, Li X, Försti A, Sundquist J, Sundquist K: Incidence of hereditary amyloidosis and autoinflammatory diseases in Sweden: endemic and imported diseases. *BMC Med Genet* 2013;14:88.
- Tanskanen M, Peuralinna T, Polvikoski T, Notkola IL, Sulkava R, Hardy J, Singleton A, Kiuru-Enari S, Paetau A, Tienari PJ, Myllykangas L: Senile systemic amyloidosis affects 25% of the very aged and associates with genetic variation in alpha2-macroglobulin and tau: a population-based autopsy study. *Ann Med* 2008;40:232–239.
- González-López E, Gallego-Delgado M, Guzzo-Merello G, de Haro-Del Moral FJ, Cobano-Marcos M, Robles C, Bornstein B, Salas C, Lara-Pezzi E, Alonso-Pulpon L, Garcia-Pavia P: Wild-type transthyretin amyloidosis as a cause of heart failure with preserved ejection fraction. *Eur Heart J* 2015;36:2585–2594.
- Pinney JH, Whelan CJ, Petrie A, Dungu J, Banyersad SM, Sattianayagam P, Wechalekar A, Gibbs SD, Venner CP, Wassef N, McCarthy CA, Gilbertson JA, Rowczenio D, Hawkins PN, Gillmore JD, Lachmann HJ: Senile systemic amyloidosis: clinical features at presentation and outcome. *J Am Heart Assoc* 2013;2:e000098.
- Janssen S, Rijswijk MH van, Meijer S, Ruinen L, van der Hem GK: Systemic amyloidosis: a clinical survey of 144 cases. *Neth J Med* 1986;29:376–385.
- Hazenber BP: Amyloidosis: a clinical overview. *Rheum Dis Clin North Am* 2013;39:323–345.
- Gafni J, Sohar E: Rectal biopsy for the diagnosis of amyloidosis. *Am J Med Sci* 1960;240:332–336.
- Westermark P, Stenkvist B: Diagnosis of secondary generalized amyloidosis by fine needle biopsy of the skin. *Acta Med Scand* 1971;190:453–454.
- van Gameren II, Hazenberg BP, Bijzet J, van Rijswijk MH: Diagnostic accuracy of subcutaneous abdominal fat tissue aspiration for detecting systemic amyloidosis and its utility in clinical practice. *Arthritis Rheum* 2006;54:2015–2021.
- Hachulla E, Janin A, Flipo RM, Saïle R, Facon T, Bataille D, Vanhille P, Hatron PY, Devulder B, Duquesnoy B: 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.
- Sjölander D, Bijzet J, Hazenberg BP, Nilsson KP, Hammarström P: Sensitive and rapid assessment of amyloid by oligothiophene fluorescence in subcutaneous fat tissue. *Amyloid* 2015;22:19–25.
- Kebbel A, Rocken C: Immunohistochemical classification of amyloid in surgical pathology revisited. *Am J Surg Pathol* 2006;30:673–683.
- Satoskar AA, Efebera Y, Hasan A, Brodsky S, Nadasy G, Dogan A, Nadasy T: Strong transthyretin immunostaining: potential pitfall in cardiac amyloid typing. *Am J Surg Pathol* 2011;35:1685–1690.
- Vrana JA, Gamez JD, Madden BJ, Theis JD, Bergen HR 3rd, Dogan A: Classification of amyloidosis by laser microdissection and mass spectrometry-based proteomic analysis in clinical biopsy specimens. *Blood* 2009;114:4957–4979.
- Lavattelli F, Vrana JA: Proteomic typing of amyloid deposits in systemic amyloidosis. *Amyloid* 2011;18:177–182.
- Jadoul M, Drüeke TB: β 2 microglobulin amyloidosis: an update 30 years later. *Nephrol Dial Transplant* 2015, Epub ahead of print.
- Noordzij W, Glaudemans AW, Longhi S, Slart RH, Lorenzini M, Hazenberg BP, Rapezzi C: Nuclear imaging for cardiac amyloidosis. *Heart Fail Rev* 2015;20:145–154.
- Fontana M, Pica S, Reant P, Abdel-Gadir A, Treibel TA, Banyersad SM, Maestrini V, Barcella W, Rosmini S, Bulluck H, Sayed RH, Patel K, Mamhood S, Bucciarelli-Ducci C, Whelan CJ, Myerson SG, Lachmann HJ, Wechalekar AD, Manisty CH, Schelbert EB, Kellman P, Gillmore JD, Hawkins PN, Moon JC: Prognostic value of late gadolinium enhancement cardiovascular magnetic resonance in cardiac amyloidosis. *Circulation* 2015;132:1570–1579.
- Karamitsos TD, Piechnik SK, Banyersad SM, Fontana M, Ntusi NB, Ferreira VM, Whelan CJ, Myerson SG, Robson MD, Hawkins PN, Neubauer S, Moon JC: Noncontrast T1 mapping for the diagnosis of cardiac amyloidosis. *JACC Cardiovasc Imaging* 2013;6:488–497.
- Noordzij W, Glaudemans AW, van Rheeën RW, Hazenberg BP, Tio RA, Dierckx RA, Slart RH: ^{123}I -labelled metaiodobenzylguanidine for the evaluation of cardiac sympathetic denervation in early stage amyloidosis. *Eur J Nucl Med Mol Imaging* 2012;39:1609–1617.

- 27 Glaudemans AW, Slart RH, Noordzij W, Dierckx RA, Hazenberg BP: Utility of ¹⁸F-FDG PET/(CT) in patients with systemic and localized amyloidosis. *Eur J Nucl Med Mol Imaging* 2013;40:1095–1101.
- 28 Kollmer J, Bendszus M, Pham M: MR neurography: diagnostic imaging in the PNS. *Clin Neuroradiol* 2015;(suppl)25:283–289.
- 29 Hazenberg BP, van Rijswijk MH, Piers DA, Lub-de Hooge MN, Vellenga E, Haagsma EB, Hawkins PN, Jager PL: Diagnostic performance of ¹²³I-labeled serum amyloid P component scintigraphy in patients with amyloidosis. *Am J Med* 2006;119:355.e15–e24.
- 30 Gertz MA, Comenzo R, Falk RH, Fermand JP, Hazenberg BP, Hawkins PN, Merlini G, Moreau P, Ronco P, Santhorawala V, Sezer O, Solomon A, Grateau G: Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France, 18–22 April 2004. *Am J Hematol* 2005;79:319–328.
- 31 Palladini G, Dispenzieri A, Gertz MA, Kumar S, Wechalekar A, Hawkins PN, Schönland S, Hegenbart U, Comenzo R, Kastritis E, Dimopoulos MA, Jaccard A, Klersy C, Merlini G: New criteria for response to treatment in immunoglobulin light chain amyloidosis based on free light chain measurement and cardiac biomarkers: impact on survival outcomes. *J Clin Oncol* 2012;30:4541–4549.
- 32 Kumar S, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK, Colby C, Laumann K, Zeldenrust SR, Leung N, Dingli D, Greipp PR, Lust JA, Russell SJ, Kyle RA, Rajkumar SV, Gertz MA: Revised prognostic staging system for light chain amyloidosis incorporating cardiac biomarkers and serum free light chain measurements. *J Clin Oncol* 2012;30:989–995.
- 33 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.
- 34 Caorsi R, Federici S, Gattorno M: Biologic drugs in autoinflammatory syndromes. *Autoimmun Rev* 2012;12:81–86.
- 35 Hakala M, Immonen K, Korpela M, Vasala M, Kauppi MJ: Good medium-term efficacy of tocilizumab in DMARD and anti-TNF- α therapy resistant reactive amyloidosis. *Ann Rheum Dis* 2013;72:464–465.
- 36 Gertz MA, Lacy MQ, Dispenzieri A, Hayman SR, Kumar SK, Leung N, Gastineau DA: Effect of hematologic response on outcome of patients undergoing transplantation for primary amyloidosis: importance of achieving a complete response. *Haematologica* 2007;92:1415–1418.
- 37 Skinner M, Santhorawala V, Seldin DC, Dember LM, Falk RH, Berk JL, Anderson JJ, O'Hara C, Finn KT, Libbey CA, Wiesman J, Quillen K, Swan N, Wright DG: High-dose melphalan and autologous stem-cell transplantation in patients with AL amyloidosis: an 8-year study. *Ann Intern Med* 2004;140:85–93.
- 38 Jaccard A, Moreau P, Leblond V, Leleu X, Benboubker L, Hermine O, Recher C, Asli B, Lioure B, Royer B, Jardin F, Bridoux F, Grosbois B, Jaubert J, Piette JC, Ronco P, Quet F, Cogne M, Fermand JP; Myélome Autogreffe (MAG) and Intergroupe Francophone du Myélome (IFM) Intergroup: High-dose melphalan versus melphalan plus dexamethasone for AL amyloidosis. *N Engl J Med* 2007;357:1083–1093.
- 39 Gatt ME, Palladini G: Light chain amyloidosis 2012: a new era. *Br J Haematol* 2013;160:582–598.
- 40 Ericzon BG, Wilczek HE, Larsson M, Wijayatunga P, Stangou A, Pena JR, Furtado E, Barroso E, Daniel J, Samuel D, Adam R, Karam V, Poterucha J, Lewis D, Ferraz-Neto BH, Cruz MW, Munar-Ques M, Fabregat J, Ikeda S, Ando Y, Heaton N, Otto G, Suhr O: Liver transplantation for hereditary transthyretin amyloidosis: after 20 years still the best therapeutic alternative? *Transplantation* 2015;99:1847–1854.
- 41 Okamoto S, Zhao Y, Lindqvist P, Backman C, Ericzon BG, Wijayatunga P, Henein MY, Suhr OB: Development of cardiomyopathy after liver transplantation in Swedish hereditary transthyretin amyloidosis (ATTR) patients. *Amyloid* 2011;18:200–205.
- 42 Ackermann EJ, Guo S, Booten S, Alvarado L, Benson M, Hughes S, Monia BP: Clinical development of an antisense therapy for the treatment of transthyretin-associated polyneuropathy. *Amyloid* 2012;19(suppl 1):43–44.
- 43 Coelho T, Adams D, Silva A, Lozeron P, Hawkins PN, Mant T, Perez J, Chiesa J, Warrington S, Tranter E, Munisamy M, Falzone R, Harrop J, Cehelsky J, Bettencourt BR, Geissler M, Butler JS, Sehgal A, Meyers RE, Chen Q, Borland T, Hutabarat RM, Clausen VA, Alvarez R, Fitzgerald K, Gamba-Vitalo C, Nocher SV, Vaishnav AK, Sah DW, Gollob JA, Suhr OB: Safety and efficacy of RNAi therapy for transthyretin amyloidosis. *N Engl J Med* 2013;369:819–829.
- 44 Hovey BM, Ward JE, Soo Hoo P, O'Hara CJ, Connors LH, Seldin DC: Preclinical development of siRNA therapeutics for AL amyloidosis. *Gene Ther* 2011;18:1150–1156.
- 45 Coelho T, Maia LF, Martins da Silva A, Waddington Cruz M, Planté-Bordeneuve V, Lozeron P, Suhr OB, Campistol JM, Conceição IM, Schmidt HH, Trigo P, Kelly JW, Labaudinière R, Chan J, Packman J, Wilson A, Grogan DR: Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. *Neurology* 2012;79:785–792.
- 46 Berk JL, Suhr OB, Obici L, Sekijima Y, Zeldenrust SR, Yamashita T, Heneghan MA, Gorevic PD, Litchy WJ, Wiesman JF, Nordh E, Corato M, Lozza A, Cortese A, Robinson-Papp J, Colton T, Rybin DV, Bisbee AB, Ando Y, Ikeda S, Seldin DC, Merlini G, Skinner M, Kelly JW, Dyck PJ; Diflunisal Trial Consortium: Repurposing diflunisal for familial amyloid polyneuropathy: a randomized clinical trial. *JAMA* 2013;310:2658–2667.
- 47 Dember LM, Hawkins PN, Hazenberg BP, Gorevic PD, Merlini G, Butrimiene I, Livneh A, Lesnyak O, Puéchal X, Lachmann HJ, Obici L, Balshaw R, Garceau D, Hauck W, Skinner M; Eprodisate for AA Amyloidosis Trial Group: Eprodisate for the treatment of AA amyloidosis. *N Engl J Med* 2007;356:2349–2360.
- 48 Kristen AV, Lehrke S, Buss S, Mereles D, Steen H, Ehlermann P, Hardt S, Giannitsis E, Schreiner R, Haberkorn U, Schnabel PA, Linke RP, Röcken C, Wanker EE, Dengler TJ, Altland K, Katus HA: Green tea halts progression of cardiac transthyretin amyloidosis: an observational report. *Clin Res Cardiol* 2012;101:805–813.
- 49 Obici L, Cortese A, Lozza A, Lucchetti J, Gobbi M, Palladini G, Perlini S, Saraiva MJ, Merlini G: Doxycycline plus tauroursodeoxycholic acid for transthyretin amyloidosis: a phase II study. *Amyloid* 2012;19(suppl 1):34–36.
- 50 Ward JE, Ren R, Toraldo G, Soohoo P, Guan J, O'Hara C, Jasuja R, Trinkaus-Randall V, Liao R, Connors LH, Seldin DC: Doxycycline reduces fibril formation in a transgenic mouse model of AL amyloidosis. *Blood* 2011;118:6610–6617.
- 51 Wall JS, Kennel SJ, Williams A, Richey T, Stuckey A, Huang Y, Macy S, Donnell R, Barbour R, Seubert P, Schenk D: AL amyloid imaging and therapy with a monoclonal antibody to a cryptic epitope on amyloid fibrils. *PLoS One* 2012;7:e52686.
- 52 Bodin K, Ellmerich S, Kahan MC, Tennent GA, Loesch A, Gilbertson JA, Hutchinson WL, Mangione PP, Gallimore JR, Millar DJ, Minogue S, Dhillon AP, Taylor GW, Bradwell AR, Petrie A, Gillmore JD, Bellotti V, Botto M, Hawkins PN, Pepys MB: Antibodies to human serum amyloid P component eliminate visceral amyloid deposits. *Nature* 2010;468:93–97.
- 53 Richards DB, Cookson LM, Berges AC, Barton SV, Lane T, Ritter JM, Fontana M, Moon JC, Pinzani M, Gillmore JD, Hawkins PN, Pepys MB: Therapeutic clearance of amyloid by antibodies to serum amyloid P component. *N Engl J Med* 2015;373:1106–1114.
- 54 van Gameren II, van Rijswijk MH, Bijzet J, Vellenga E, Hazenberg BP: Histological regression of amyloid in AL amyloidosis is exclusively seen in patients with a complete response of serum free light chain. *Haematologica* 2009;94:1094–1100.