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Abstract

Hazenberg BPC. Clinical approach of patients with systemic amyloidosis

Amyloidosis is the name of diseases characterised by deposition of protein fibrils with a beta-sheet structure. This beta-sheet structure generates affinity of amyloid for Congo red dye and is resistant to proteolysis. The main three types of systemic amyloidosis are AA (related to underlying chronic inflammation), AL (related to underlying monoclonal light chain production), and ATTR amyloidosis (related to old age or underlying hereditary mutations of transthyretin). Signs and symptoms vary among the three types and the treatment is different for each type. If a patient is suspected to have systemic amyloidosis, proof of the presence of amyloid in tissue must be obtained first and systemic involvement should be unequivocal. Determination of the precise type of amyloid is extremely important and one should start to detect the particular amyloid precursor. Assessment of size and function of vital organs and tissues is essential in the clinical work-up of a patient with systemic amyloidosis. A fast and thorough clinical evaluation is necessary to obtain all relevant information for prognosis and choice of treatment. The treatment is based on the "precursor-product" concept, in which the supply of amyloid precursor is the rate limiting step for further accumulation of amyloid. Effective therapy quickly and completely stops ongoing supply of precursor. In this respect, investigations such as serum amyloid P component (SAP) scintigraphy may help not only to investigate organ involvement but also response to treatment. The effects of therapy on both underlying disease and amyloidosis should be monitored frequently during follow-up.

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Introduction

Amyloidosis is the name of a group of diseases characterised by deposition of proteinaceous fibrils with a molecular ß-sheet structure (1). This structure of the fibrils is responsible for its insolubility, resistance to proteolysis, and binding affinity for Congo red dye and the consequent green birefringence with polarised light. Amyloid fibrils are derived from a variety of protein precursors. The extracellular deposition of amyloid

fibrils in organs and tissues results in loss of function and often causes prominent swelling of the affected organ or tissue. Deposition of amyloid can be localised (produced in and limited to one organ or site of the body) or systemic (deposition in various organs and tissues throughout the body). The precursor protein is used for typing amyloid in the current classification of amyloidosis (2). Signs and symptoms of systemic amyloidosis differ among the various types of amyloidosis (1-3). The aim of this article is to provide a clinical overview of systemic amyloidosis: an approach to diagnosis, clinical evaluation, and background of therapy.

Systemic amyloidosis

Localised deposition of amyloid plays a still unresolved role in widespread diseases such as Alzheimer's disease (ß-protein in the plaques) and diabetes mellitus type II (amylin in the islands of Langerhans). Systemic deposition of amyloid, however, is directly related to the grim prospects of systemic amyloidosis. Three major types can be distinguished (1-3). AA amyloidosis is caused by longstanding inflammation. Serum amyloid A protein (SAA), an acute phase reactant, is the precursor. Signs of kidney disease, such as proteinuria (progressing to nephrotic syndrome) and loss of renal function (progressing to renal failure), are observed most frequently (in about 90 % of cases). Less frequent manifestations are autonomic neuropathy, splenomegaly, hepatomegaly, goiter, and cardiomyopathy.

AL amyloidosis is caused by an, often low-grade, plasma cell dyscrasia. Lambda or kappa immunoglobulin light chain is the precursor of this type of amyloid. Clinical manifestations are diverse, such as cardiomyopathy, hepatomegaly, splenomegaly, nephrotic syndrome, renal failure, orthostatic hypotension, diarrhea, peripheral and autonomic neuropathy, arthropathy, carpal tunnel syndrome (CTS), and glossomegaly. The diversity of disease manifestations is related to severity of deposition in the various organs and tissues.

ATTR amyloidosis is caused by many autosomal dominantly inherited point mutations of the precursor protein transthyretin (TTR). Transthyretin is the acronym of transport protein of thyroid hormone and retinol binding protein. About 100 of these TTR mutations have been described, but the so-called TTR-Met30 mutation is seen most frequently. Prominent clinical manifestations are familial peripheral and autonomic neuropathy, but cardiomyopathy, renal failure, and eye involvement (vitreous opacities) are also often observed in the course of the disease. Severe cardiomyopathy is the

presenting manifestation in some TTR mutations. In very old age, non-mutated ("wild-type") TTR can also act as amyloid precursor by a still unknown mechanism. This "wild-type" ATTR amyloidosis (formerly called senile systemic amyloidosis) is characterised by a slowly progressive cardiomyopathy.

A fourth type is Aβ_2M amyloidosis that is associated with renal failure and longstanding (i.e. at least 5-10 years) dialysis with decreased clearance and elevated serum levels of beta-2-microglobulin (β_2M).β_2M is the precursor of this type of amyloid. Clinical manifestations are predominantly arthropathic, such as tenosynovitis, shoulder pain, CTS, periarticular cysts, pathological fractures, and destructive spondyloarthropathy. Synovial tissue biopsy is the method to detect this type of amyloid. Kidney transplantation stops the disease (4, 5). Aβ_2M amyloidosis is a disabling disease that should be recognised and treated, but can easily be distinguished from the main three types (AA, AL, and ATTR) of systemic amyloidosis because of the association with dialysis. The other three types are often difficult to diagnose, show variable involvement of many organs and tissues, and are challenging in finding the most appropriate treatment.

Histology is essential for diagnosis
The diagnosis of amyloid is based on showing its presence in tissue. Method of choice is a positive Congo red-stained tissue specimen showing the characteristic apple-green birefringence in polarised light (figure 1). Aspiration of a subcutaneous fat sample of the abdominal wall is the most elegant and least inconvenient method for this purpose, with a sensitivity ranging between 54% (6) and - in experienced

hands - 93% (7) with a corresponding specificity of 100% (7). These figures are comparable with those of the well-known rectum biopsy (7, 8). In case one of the primary biopsy sites (fat or rectum) is negative for amyloid and suspicion of amyloidosis remains strong, a biopsy of the alternate site is useful to increase the chance of detecting amyloid. A bone marrow biopsy can also be used, but has a rather low sensitivity of 50-60% (7, 8). If all screening biopsies are negative but suspicion of amyloidosis remains strong, a biopsy of the affected organ or tissue is indicated (1, 7, 9).

Localised or systemic deposition of amyloid
It is important to establish whether deposition of amyloid is localised or systemic. Some sites of the body are almost exclusively involved in systemic amyloidosis, such as kidneys, liver, nerves, abdominal fat, and spleen. If such a site is positive for amyloid, one may conclude to systemic amyloidosis. Localised amyloid can often be found in some other specific sites of the body (such as eyelid, cardiac atria, larynx, ureter, skin, etc.). If in these cases amyloid is not detected elsewhere in the body one may conclude to localised amyloidosis. Most other sites (bone marrow, heart, bowel, lung, joint, etc.) are nearly always involved in systemic amyloidosis, but can be localised occasionally. In this situation it is necessary to demonstrate histological and/or clinical presence of amyloid in two different organs or tissues. For this demonstration, however, it is sufficient to have histological proof at one site (such as bone marrow, skin, or rectum) and typical clinical involvement (such as nephrotic syndrome, hepatomegaly, macroglossia, or cardiomyopathy) at the other site (10).
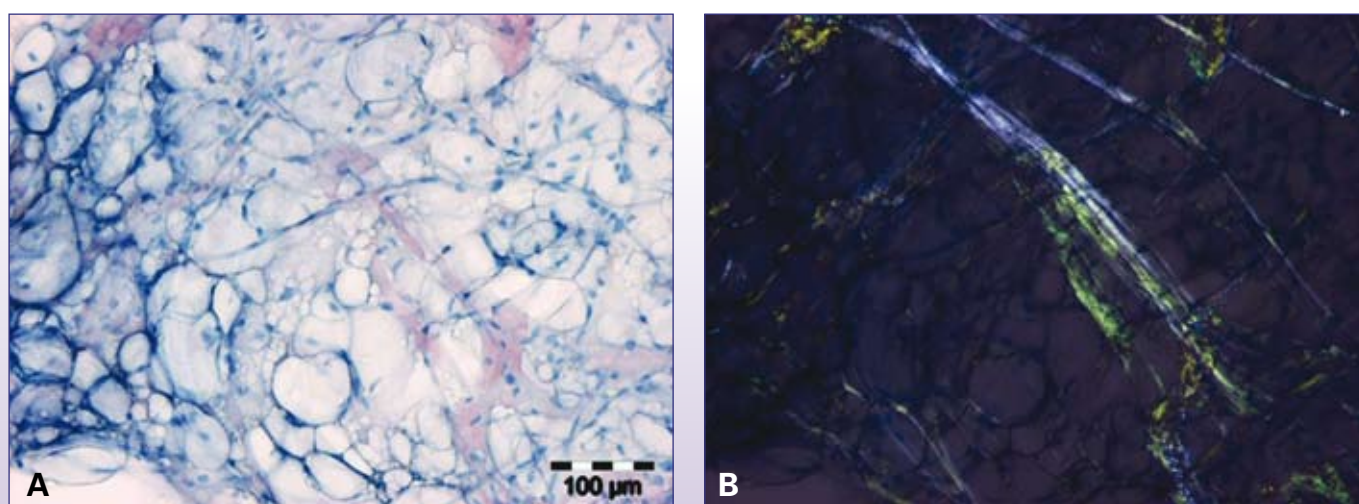

Figure 1. Example of an abdominal subcutaneous fat aspirate, stained with Congo red.
A. Viewed in normal light: amyloid is stained red. Bar length is 100 μm.
B. The same specimen viewed in polarised light: amyloid shows apple-green birefringence (collagen is bluish-white).

Typing amyloid with confidence

Determination of the type of amyloid is very important. In the majority of cases the type of amyloid can be suspected from medical history and clinical picture. Amyloidosis in a patient with longstanding rheumatoid arthritis and nephrotic syndrome points to AA type. A patient with polyneuropathy who is member of a family with hereditary ATTR amyloidosis most probably also suffers from this disease. And for a patient with characteristic shoulder pads and glossomegaly it is hard to believe in a type different from AL amyloidosis. Nevertheless, even in patients with strong clinical evidence for a particular type of amyloid it is a good habit to search for solid confirmation of the specific type of amyloid involved. The clinical consequences of incorrect typing of amyloid can be huge, because prognosis and therapy of the three major types of systemic amyloidosis differ so much. Immunohistochemistry of a biopsy is common practice by typing amyloid using specific antibodies. In AA amyloidosis this technique is sufficient, provided that sensitive and specific monoclonal antibodies are used, such as mc1 (10) or Reu.86.2 (11, 12). But in ATTR amyloidosis and especially in AL amyloidosis immunohistochemistry is less reliable than in AA amyloidosis (13). The false positive and false negative results may be due to heterogeneity of amyloid deposits, loss of epitopes in the fibril structure, lower sensitivity and specificity of (polyclonal) antibodies, and non-specific adherence of immunoglobulins to amyloid deposits or the background (10). One should realise that lack of a positive family history does not exclude ATTR amyloidosis as shown by a considerable number of "sporadic" cases that have been described (14). Therefore the presence of a TTR mutation must be confirmed by DNA analysis in all cases of ATTR amyloidosis. The only exclusion of this requirement is a slowly progressive amyloid cardiomyopathy at old age that is typical of "wild-type" ATTR amyloidosis.

In patients with AL amyloidosis a monoclonal plasma cell dyscrasia with overproduction of lambda or kappa light chain will be present. It can be detected in bone marrow (clonal dominance by immunophenotyping of plasma cells), urine (Bence Jones proteins, immunofixation of concentrated urine), and blood (M-protein, immunofixation, and – most important of all – by the free light chain assay). However, a monoclonal gammopathy of undetermined significance (MGUS) is frequently present in healthy older persons, about 2-4% in persons over 50 years old and even higher with advancing age (15). One should therefore realise that detection of an MGUS does not exclude other types than AL amyloidosis. It is important to notice that the clinical picture of ATTR amyloidosis and AL amyloidosis may be similar, such as in cases with polyneuropathy, autonomic neuropathy, cardiomyopathy, and carpal tunnel syndrome. In a patient with such a clinical picture it is therefore not sufficient to show the presence of a plasma cell dyscrasia but also necessary to exclude a TTR mutation before one can diagnose AL amyloidosis (14).

Immuno-electron microscopy seems to be more specific for typing all types of amyloid (16). A promising development for typing amyloid with confidence is proteomics (17). This development is especially useful to distinguish between AL and ATTR amyloid. Techniques such as two-dimensional (2D) polyacrylamide gel electrophoresis followed by matrix-assisted laser desorption/ionization mass spectrometry and peptide mass fingerprinting (18) and laser microdissection and mass spectrometry (19) have high sensitivity with corresponding high specificity. Currently these sophisticated, expensive, and time-consuming techniques are only available in highly specialised centres, so immunohistochemistry remains standard for typing amyloid in daily practice.

Amyloid precursor

After typing amyloid one should look for an amyloid precursor in the blood. Detection of such a precursor and measuring its serum concentration is important for the choice of treatment. In AA amyloidosis the precursor is SAA, an acute phase protein (20). The behaviour of SAA during inflammation is similar to C-reactive protein (CRP), a protein that is used in daily practice. In ATTR amyloidosis the precursor is mutated TTR, which protein can be detected by isoelectric focusing (21). In AL amyloidosis the free light chain assay is used to quantify serum levels of free lambda and kappa precursor proteins using specific antibodies raised against epitopes that are hidden in the complete immunoglobulin (22).

Clinical evaluation

It is useful to obtain a clinical overview of the "amyloid load", i.e. affected organs and tissues and severity of amyloid deposition in vital organs (such as heart, liver, and kidneys). One should not forget to ask for family history, impotence, orthostatic complaints, loss of sensibility, fatigue, weight loss, and bowel problems. Physical examination should also focus on signs such as orthostatic blood pressure, friability of skin, glossomegaly, arthropathy, hepatomegaly, splenomegaly, oedema, cardiac failure, and loss of sensibility and muscle strength of extremities.

A thoughtful systematic clinical approach is indicated. The heart can be examined with electrocardiography (signs of low voltage and pseudo-anteroseptal infarction), chest X-ray (normally sized heart despite signs of cardiac failure), echocardiography (thickness of septum and ventricular walls), MRI (wall thickness, delayed contrast-enhanced imaging), 24 hour Holter registration (conduction, rhythm, and heart rate variability) and a MUGA scan (left ventricle ejection fraction). NT-proBNP and troponin levels in blood are extremely useful for assessing cardiac involvement and for risk evaluation (23-25). The kidneys can be examined with serum albumin, creatinine clearance, urine sediment and proteinuria. The liver can be examined with serum albumin, liver enzymes such as alkaline phosphatase, bilirubin, coagulation tests, and cholinesterase. Thyroid stimulating hormone can be used for the thyroid and fasting cortisol for the adrenal glands.

Autonomic function tests ("Ewing battery") and heart rate variability can be used for evaluation of autonomic neuropathy (26, 27). Electromyography can be used to assess peripheral neuropathy. Abdominal ultrasound is useful to evaluate size and echogenicity of liver, spleen and kidneys. Not all of the examinations mentioned above need to be used, because often it is obvious that clinical organ involvement is not present at all. However, echocardiography should be considered in all patients, even in those without cardiac symptoms. The role of nuclear medicine techniques in diagnosis and clinical evaluation, such as serum amyloid P component (SAP) scintigraphy (28-31), will be discussed in other articles of this issue.

In 2004 at a consensus meeting in Tours, the amyloid community agreed upon guidelines for organ involvement, haematological response criteria, and organ response criteria that are currently used in AL amyloidosis (32). Table 1 shows the consensus criteria for organ involvement.

Prognosis

Prognosis is poor if the underlying precursor production remains untreated. The prognosis depends upon the type of amyloid, the severity of amyloid deposition, the number of vital organs affected, the presence of symptomatic cardiomyopathy, the severity of the associated disease, and the response to therapy of the underlying precursor-producing process. Patients with untreated AL amyloidosis have the worst prognosis, with a median survival of less than one year (1, 9). Median survival in untreated AL amyloidosis in case of symptomatic cardiomyopathy is 4-6 months, in case of kidney involvement about 2 years, and in case of CTS more than 3-4 years. Untreated patients with AA amyloidosis have a median survival of 2-4 years (1, 9). Survival in AA strongly depends upon the activity of the underlying inflammation (33). Patients with untreated ATTR amyloidosis may survive up to 10-15 years, but median survival is between 5 and 10 years (1).

Treatment

The foundation of treatment is the so-called "precursor-product" concept (34). Central idea of this concept is that further growth of amyloid deposits will stop when the supply of necessary precursors is put to a stop. In AA amyloidosis treatment is aimed at decreasing SAA serum levels to normal basal values (below 3 mg/l). This aim can only be achieved by a complete suppression or eradication of the underlying chronic inflammation. Examples are surgical treatment of chronic osteomyelitis and antibiotic treatment of infectious diseases such as tuberculosis and leprosy. In

Organ	Criterion
Kidney	24-hr urine protein >0.5 g/day, predominantly albumin
Heart	Echo: mean wall thickness >12 mm, no other cardiac cause
Liver	Total liver span >15 cm in the absence of heart failure or alkaline phosphatase >1.5 times institutional upper limit of normal
Nerve	Peripheral: clinical; symmetric lower extremity sensorimotor peripheral neuropathy Autonomic: gastric-emptying disorder, pseudo-obstruction, voiding dysfunction not related to direct organ infiltration
Gastrointestinal tract	Direct biopsy verification with symptoms
Lung	Direct biopsy verification with symptoms Interstitial radiographic pattern
Soft tissue	Tongue enlargement, clinical Arthropathy Claudication, presumed vascular amyloid Skin Myopathy by biopsy or pseudohypertrophy Lymph node (may be localized) Carpal tunnel syndrome

Table 1. Organ involvement: biopsy of affected organ or biopsy at an alternate site*

*Alternate sites available to confirm the histologic diagnosis of amyloidosis: fine-needle abdominal fat aspirate and/or biopsy of the minor salivary glands, rectum, or gingiva. Derived from Gertz et al (32).

chronic inflammatory diseases such as rheumatoid arthritis and Crohn's disease effective suppression of inflammation (resulting in a substantial decrease of serum SAA levels below 3 mg/l) is often difficult, but should be attempted (33). To achieve this goal, cytostatic drugs can be used (such as methotrexate and azathioprine), but also biologicals such as anti-TNF (tumour necrosis factor) drugs (such as infliximab, adalimumab and etanercept). In patients with TRAPS (TNF-Receptor-Associated Periodic Syndrome), etanercept (acting as soluble TNF receptor) seems to be a rational treatment because of the abnormal function of the mutated TNF receptor. The interleukin-1-receptor antagonist anakinra is often highly effective in cryopyrin-related diseases such as familial cold urticaria and Muckle-Wells syndrome (35). Colchicine has a central place in the treatment of Familial Mediterranean Fever (FMF), not only by reducing the frequency and severity of attacks, but also by preventing the development of AA amyloidosis (36).

In AL amyloidosis the aim of treatment is to eradicate the underlying plasma cell dyscrasia by chemotherapy. High dose melphalan with autologous stem cell transplantation is favourable in a group of well-selected patients (37), but currently less toxic and less intensive regimens are studied using novel drugs such as thalidomide, lenalidomide and bortezomib, all combined with dexamethazone (38). In patients with hereditary ATTR amyloidosis liver transplantation is the only possibility to remove the source of 99% of the mutated TTR in the circulation (39). However, this approach is not always successful, because amyloid sometimes still progresses in the heart after transplantation (40, 41). Beside treatment aimed at the underlying disease, it is necessary to give supportive treatment for loss of organ function that is caused by amyloid deposition. Multisystem involvement often results in a mixture of serious problems and in such a situation it is almost impossible to realise an appropriate treatment for all symptoms (1).

Monitoring the effect of treatment

Measuring the effect of treatment is important for such an intangible disease as systemic amyloidosis. The expectation is that no further accumulation of amyloid deposits will occur after successful elimination of the precursor supply. Besides, the body itself will possibly try to remove amyloid. Repeated measurements will help to get an impression of the treatment effect. Two different processes should be monitored in this way.

Firstly, the underlying precursor-producing process with the respective precursors: serum SAA, free kappa or lambda light chain, and mutated ATTR in AA, AL, and ATTR amyloidosis respectively. If treatment is successful SAA levels should fall below 3 mg/l, free kappa and lambda levels and the kappa/lambda ratio should return to the reference ranges, and mutant TTR should not be detectable in the blood anymore. Secondly, there is the process of amyloid accumulation, measuring the clinical "amyloid load". For this measurement

quantitative abnormal clinical signs should be monitored, such as serum albumin, alkaline phosphatase, bilirubin, NT-proBNP, troponin, creatinine clearance, proteinuria, ventricular wall thickness, left ventricle ejection fraction, conduction and rhythm, heart rate variability, Ewing battery results, and the sizes of enlarged organs, such as liver, spleen, and kidneys. The abdominal subcutaneous fat aspiration can be repeated at each time point to get an idea of the severity of the presence of amyloid or its disappearance from tissue (41-43). SAP scintigraphy, if abnormal at presentation, can also be used to monitor amyloid regression in the individual patient (44, 45). At the consensus meeting in Tours in 2004 also a set of response criteria in systemic AL amyloidosis has been accepted (32).

Treatment perspectives

The "precursor-product" concept focuses on stopping ongoing deposition of amyloid. Clinical research is focused on developing new drugs that interfere with amyloid deposition or stimulate removal of amyloid deposits. Currently new drugs are investigated that stabilise TTR in the circulation and hamper deposition of amyloid, such as diflunisal and tafamidis. Both drugs stabilise in vitro the TTR tetramer in blood and prohibit its degradation into amyloidogenic dimers and monomers (46). However, results of clinical trials have not been published yet. Doxycyclin stimulates removal of ATTR amyloid deposits in mice (47). A promising drug for patients with AA amyloidosis is eprodisate (48). This drug prohibits binding of SAA to glycosaminoglycans in tissue (49). CPHPC is a drug that leads to depletion of SAP from the circulation (50). This mechanism possibly stops accumulation of amyloid and may be useful for all types of systemic amyloidosis (51). A completely different approach is vaccination. Early research was focused on conformational epitopes present in all types of amyloid that might be used for vaccination (52). The London group recently demonstrated in mice that CPHPC followed by anti-SAP antibodies resulted in a quick removal of almost all AA amyloid from the tissues (53). If this huge effect on amyloid turns out to be valid in human beings, it might dramatically change the prospects of patients with all types of systemic amyloidosis.

Conclusion

A systematic evaluation of patients with systemic amyloidosis helps to get a grip on this intangible disease. Histological proof of amyloid, verification of systemic involvement, determination of the particular type of amyloid and its precursor form the background of a thoughtful clinical evaluation. New techniques such as ^{123}I-SAP scintigraphy may have a place in this evaluation. The "precursor-product" concept is still the current basis of treatment, but research is aimed at finding new ways to attack amyloid.

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