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EDITORIAL

Systemic amyloidosis: are we moving ahead?

G. Merlini

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ABSTRACT

Systemic amyloidoses are a wide group of diseases with different courses, treatments and prognoses. Unequivocal typing of amyloid deposits is important for correct diagnosis and appropriate treatment. At present, the most effective therapeutic approach is based on eliminating the supply of amyloidogenic precursor. New effective therapies will stem from our improved knowledge of the molecular mechanisms of amyloidosis.

Amyloidosis is a disease in which extracellular protein misfolding has a prominent role.[1] Localised or systemic deposition of protein fibrils with a β-sheet structure is the lowest common denominator of a wide group of diseases with different causes, courses, treatments and prognoses. The differential diagnosis of the various types may be beset with difficulties. In this issue, Hazenberg and colleagues propose a rationale step-by-step approach to the three major types of systemic amyloidoses.[2] These authors appropriately underline the paramount importance of correctly typing the amyloid deposits because this dictates both prognosis and treatment. Although genetic testing is necessary for an appropriate work-up of patients with amyloidosis,[3] unequivocal characterisation of amyloid deposits remains essential. Optical microscopy immunohistochemistry satisfactorily types reactive amyloidosis (AA) deposits, although it has several limitations in typing other amyloidoses. To overcome these difficulties, Arbustini *et al.* developed an electron microscopy immunohistochemistry method.[4] This method, which is feasible in major hospitals, unambiguously characterises amyloid deposits by demonstrating the typical ultrastructural features of the amyloid fibrils and, using specific antibodies, by co-localising the amyloidogenic protein with the fibrils.[5] Methods for extracting and chemically characterising the fibril proteins have been proposed,[6,7] but they require expertise and resources available in only a few centres worldwide. Treatment design also requires a high level of expertise. Currently, the most effective approach is the so-called 'precursor-product' concept, based on eliminating the supply of amyloidogenic precursor. This can sometimes be achieved easily, such as in colchicine treatment of most patients with familial Mediterranean fever. However, in amyloidosis caused by transthyretin variants, the optimal timing of liver transplantation and the possible need to co-transplant other organs, such as kidney and heart, irreversibly damaged by amyloid deposition, demand experience. The amyloidosis caused by monoclonal immunoglobulin light chains (AL) represents a difficult therapeutic challenge. It is necessary to achieve a fine balance between the need to annihilate the amyloidogenic plasma cell clone and the capacity of fragile amyloid patients to bear toxic chemotherapy. The chemotherapy approach was largely adapted from that used for multiple myeloma and includes high-dose melphalan followed by autologous stem cell rescue. It soon became clear that this produced intolerable treatment-related mortality and selection criteria were proposed.[8] This is also the case for less aggressive regimens such as VAD (vincristine, doxorubicin and dexamethasone). As reported by Van Gameren and colleagues in this issue, vincristine often has to be omitted because of peripheral and autonomic neuropathy, and doxorubicin because of its potential cardiac toxicity.[9] The dexamethasone scheduled in the

VAD regimen can also be too toxic for frail amyloid patients. We, therefore, developed a modified high-dose dexamethasone regimen which produced 35% responses in a median time of four months without significant toxicity.[10] Adding melphalan to the high-dose dexamethasone improved the response rate to 67% with 33% complete remissions without significant toxicity.[11] These results were achieved in patients who were ineligible for stem cell transplantation because of advanced organ involvement, making this regimen a viable alternative to autologous stem cell transplant. Van Gameren *et al.* also deal with the important issue of determining criteria for organ involvement and response to therapy.[9] Indeed, these criteria must be defined in order to facilitate comparison of populations of amyloid patients and effects of therapies. An ongoing international initiative to define such criteria should be finalised during the Xth Symposium on Amyloidosis in France in April 2004.

Better understanding of the molecular mechanisms of amyloidosis has led to new treatment approaches attacking different steps of the amyloidogenic cascade.[1] A few drugs aimed at mobilising amyloid deposits are under investigation. One of these, 4'-iodo-4'deoxydoxorubicin,[12] developed by our group, awaits better definition of its schedule and possible integration into a comprehensive therapy approach to amyloid disease. The pivotal mechanism underlying cellular damage caused by the amyloid process is being intensively investigated. Its clarification will have a strong impact on redirecting the search for new drugs. We are moving ahead: improved care of this complicated disease will be achieved by dismantling the amyloidogenic process through the integrated use of several drugs presently under development.[13] This will put the emphasis on early diagnosis, to be able to treat these patients before the amyloid process has irreversibly damaged organ function. The protean clinical presentation of amyloidosis requires a high level of alertness by physicians. While differential diagnosis and treatment necessitate the expertise of specialised centres, timely identification of these patients depends on education, which requires appropriately funded concerted initiatives.

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REVIEW

Therapeutic options in systemic AL amyloidosis

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ABSTRACT

Systemic amyloid light chain (AL) amyloidosis is a severe disease with unfavourable prognosis. Since the late 1970s different therapeutic modalities in AL amyloidosis have been investigated, trying to prolong survival. This review deals with the therapeutic modalities in AL amyloidosis to date, and highlights future perspectives.

INTRODUCTION

Systemic amyloidosis is the name of a group of multisystem diseases caused by deposition of insoluble fibrils in organs and tissues, leading to organ dysfunction. Depending on the type of precursor protein of the amyloid fibril, different forms of systemic amyloidosis are recognised.[1] The four main systemic types are shown in *table 1*. AA amyloidosis and AL amyloidosis are the two most frequent.

In AL amyloidosis the precursor protein is a monoclonal immunoglobulin kappa or lambda light chain. In rare cases AL amyloid deposits are localised (e.g. larynx, eyelid, urinary tract) and the underlying monoclonal plasma cell proliferation is thought to be closely connected to the same site. This localised AL amyloidosis is self-limiting and should be treated with local surgery. However, systemic deposition of AL amyloid is far more frequent and in this situation the underlying monoclonal plasma cell dyscrasia is localised in the bone marrow. The plasma cell dyscrasia is often low grade and usually lacks the malignant sheets of immature plasma cells in the bone marrow as in multiple myeloma (MM). However, the accumulation of amyloid fibrils in vital organs leads to organ failure and death. The prognosis of untreated AL amyloidosis is worse than uncomplicated multiple myeloma. Kyle *et al.* found a median survival in untreated AL amyloidosis of 13 months from the time of diagnosis, with considerably shorter

Table 1

The four major types of systemic amyloidosis based on precursor protein[1] - clinical association and results of tissue analysis with Congo red stain and immunohistology

TYPE	ASSOCIATION	CONGO RED	IMMUNOHISTOLOGY				
			SAP	κ/λ	SAA	β_2M	TTR
AL	Plasma cell dyscrasia	+	+	+	-	-	-
AA	Chronic inflammation	+	+	-	+	-	-
Aβ_2M	Chronic dialysis	+	+	-	-	+	-
ATTR	Familial	+	+	-	-	-	+

SAP = serum amyloid P component, κ = kappa light chain, λ = lambda light chain, SAA = serum amyloid A protein, β_2M = β_2-microglobulin, TTR = transthyretin.

median survival for patients with congestive heart failure.[2] The incidence of AL amyloidosis is low (nine per million a year), the median age is 64 years, and it is more frequent in men (male : female = 3:2).[3]

DIAGNOSIS AND CLINICAL EVALUATION

Diagnosing AL amyloidosis can be difficult due to the insidious nature of this disease, the low incidence, and the various individual clinical features. The main organs involved are the heart, kidneys, liver, and peripheral and autonomic nervous system. Deposition of amyloid in the heart leads to restrictive cardiomyopathy and primary right-sided heart failure, in the kidneys it causes proteinuria and/or renal insufficiency, and in the liver it leads to enlargement and typically to a rise in alkaline phosphatase. Amyloid deposits in the peripheral and autonomic nervous system cause classic polyneuropathy and autonomic neuropathy (e.g. orthostasis). Other features include gastrointestinal tract involvement (leading to constipation, diarrhoea, and/or bleeding), arthropathy, carpal tunnel syndrome and haemorrhagic diathesis. Almost pathognomonic features are macroglossia and periorbital purpura; however, these are present in only 8% of cases. Nearly all patients suffer from malaise and weight loss.

Diagnosis can be made by staining biopsy material with Congo red, giving characteristic apple-green birefringence of eosinophil (red) amorphous material when viewed in polarised light. The most convenient site for biopsy is subcutaneous abdominal fat tissue, aspiration of this fat tissue being a bedside or outpatient procedure of minute's duration. Kyle *et al*. showed that in AL amyloidosis the percentage of positive fat aspiration was 80%. This is comparable with the 75% positive biopsy rate in classic rectum biopsy, a more invasive procedure.[2]

When amyloid is detected, the type of amyloidosis must be established. In AL amyloidosis this can be done by immunofixation of serum and urine to detect kappa or lambda light chain overproduction. Due to the usual low grade of plasma cell dyscrasia this investigation is negative in 10% of patients.

Bradwell *et al*. recently developed a highly sensitive quantitative immunoassay for serum free light chains. This test is much more sensitive for detecting immunoglobulin free light chain than urine testing, and can be used for diagnosis and follow-up after therapy.[4-6] However, care must be taken as patients with non-AL amyloidosis may test false-positive.

Immunophenotyping of plasma cells in bone marrow (kappa : lambda ratio >3 or <1) also points to the presence of a monoclonal gammopathy. In about 40% of cases, immunohistochemical staining (kappa and lambda) of a Congo red positive tissue specimen may provide additional information regarding the AL nature of the amyloid involved.[7]

At diagnosis, patients often suffer from advanced amyloidosis with involvement and dysfunction of many organs. This limits the therapeutic options. Therefore, once diagnosis of AL amyloidosis has been confirmed, possible organ involvement and organ dysfunction has to be thoroughly investigated with special attention for vital organs. Criteria of clonal and clinical involvement and response to therapy have been proposed by leading groups.[8-10] The most relevant criteria are depicted in *table 2*. An example of classic low-voltage electrocardiography in amyloid cardiomyopathy is shown in *figure 1*.

The extent of organ involvement and organ dysfunction as well as clinical parameters of function, such as WHO performance score and NYHA (New York Heart Association) score of heart failure, are important factors that will be investigated in the current Dutch national HOVON 41 protocol for patients with AL amyloidosis. In Groningen, all patients are also evaluated by serum amyloid P component (SAP) scintigraphy. Radiolabelled SAP is a specific tracer for amyloid and as the localisation of labelled SAP to amyloid does not depend on active deposition it can be used as a quantitative method for imaging amyloid deposits *in vivo*. SAP scintigraphy has a 90% diagnostic sensitivity in AL amyloidosis and is the only method available for serially monitoring amyloid throughout the body.[11-13] *Figure 2* shows an example of such a series in one of our patients.

THERAPEUTIC OPTIONS

Precursor-product concept

Chemotherapy

A rational therapeutic goal is to reduce or eliminate the precursor protein production, prohibiting further deposition of amyloid fibrils in organs and thereby preventing deterioration of organ function. Given the hypothesis that amyloidosis is a dynamic process of deposition and removal, resolution of amyloid deposits may be expected, enlarged organs may shrink to normal size, and organ function may be restored.[11]

In AL amyloidosis the precursor protein is a monoclonal immunoglobulin light chain. Therefore, the use of chemotherapy, analogous to chemotherapy in MM, is a rational approach.

Alkylating agents such as oral melphalan in combination with prednisolone prolonged survival from 6 to 9 months to 16 to 18 months in a subgroup of patients with AL amyloidosis compared with patients treated with colchicine only.[8,10] Oral melphalan and prednisolone is

Table 2

Assessment of vital organ involvement and clonality in AL amyloidosis - some criteria for clinical and clonal responses after therapy

	METHOD OF INVESTIGATION	INVOLVEMENT	RESPONSE
Organ			
Heart	Heart failure history (NYHA I-IV)	NYHA II-IV	Decrease of two classes
	Electrocardiography	Low voltage pattern	
	Echocardiography	Septum thickness >11 mm	Decrease of 2 mm thickness or below 12 mm
	MUGA scan	Ejection fraction <45%	Increase of 20%
	24-hour electrocardiography	Conduction, rhythm, HRV	
Liver	Abdominal ultrasound	Hepatomegaly (span >15 cm)	>2 cm decrease liver span
	Alkaline phosphatase	AP >200 U/l	>50% decrease of AP
	Bilirubin		
Kidney	Creatinine clearance	Clearance <90 ml/min	
	Proteinuria	Proteinuria >0.5 g/day	>50% decrease of proteinuria*
	Serum albumin	Albumin <30 g/l	Increase of >10 g/l
Neuropathy	Clinical examination	Abnormal examination or test	Test response
	Electromyography		
	Orthostatic hypotension		
	Autonomic function test†		
Clonal			
	Serum and urine immunofixation		PR: >50% reduction of clonal protein in serum and urine*
	Bone marrow biopsy	MM or MGUS	CR: disappearance of all signs of monoclonality§
	Immunophenotyping	Monoclonality	
	Immunoassay free light chain		

NYHA = New York Heart Association scale of heart failure, MUGA = multigated blood pool scan, HRV = heart rate variability, AP = alkaline phosphatase, PR = partial response, CR = complete response, MM = multiple myeloma, MGUS = monoclonal gammopathy of unknown significance.
**Without being explained by diminishing protein excretion caused by progressive renal failure, †using validated tests based on Ewing and Clark, §no monoclonal protein in serum and urine and normalisation of number of plasma cells (<5%) with a normal kappa:lambda ratio in bone marrow.*

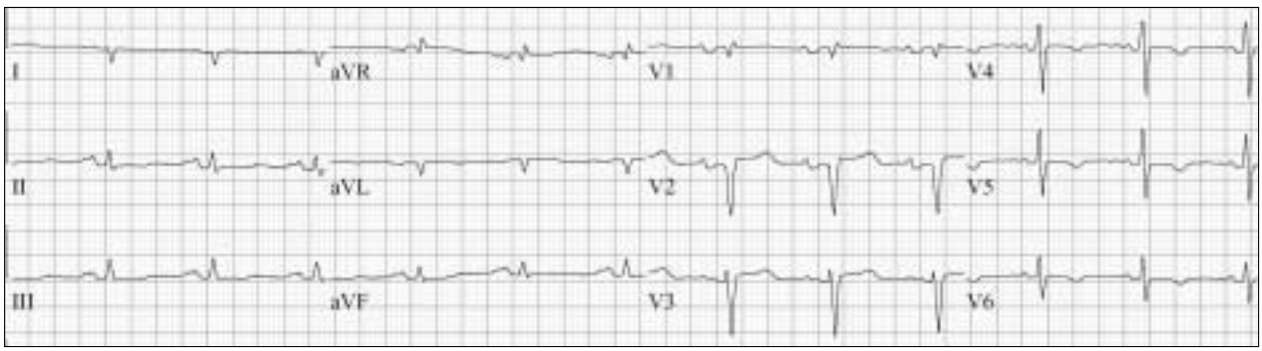
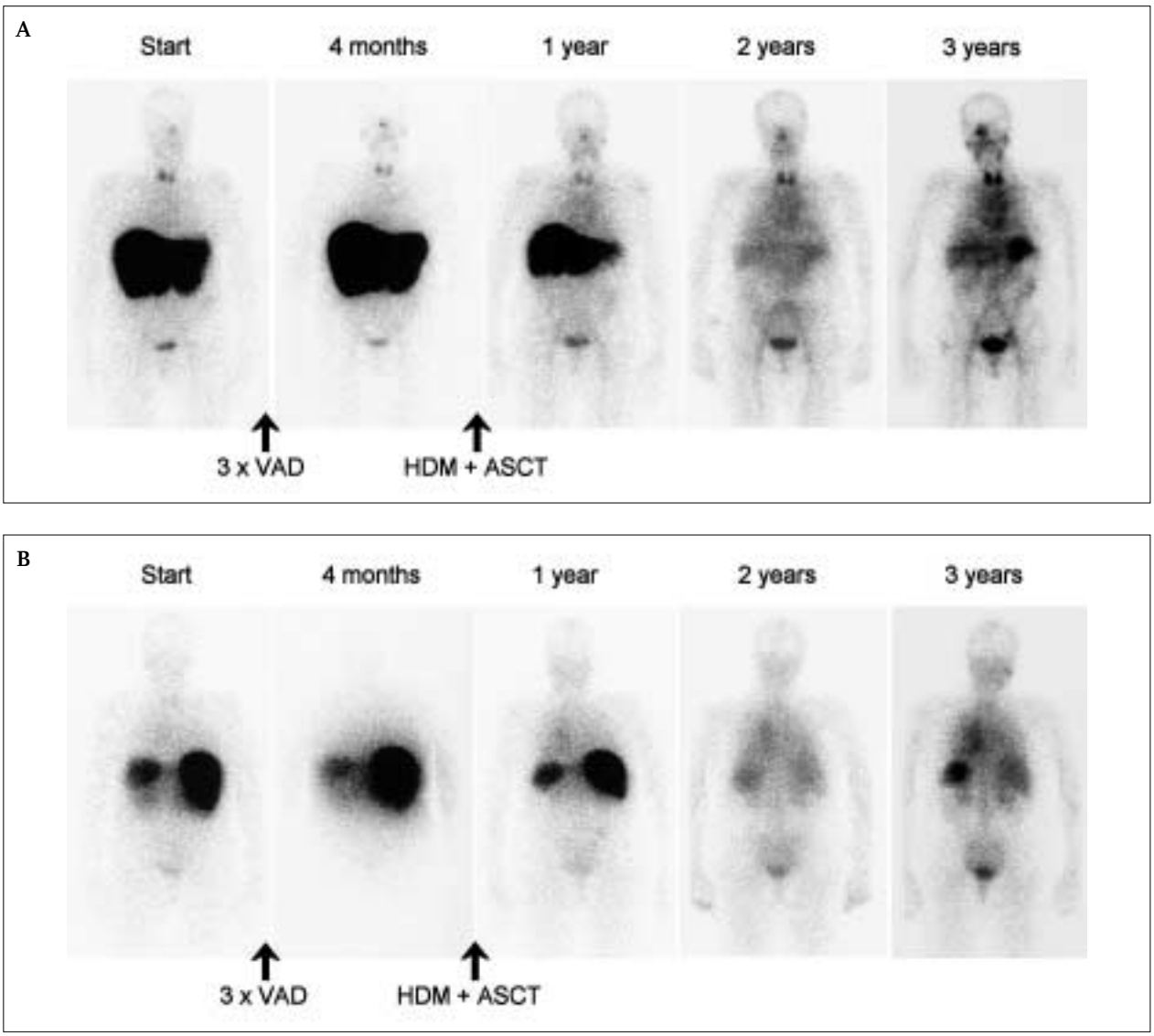


Figure 1

Classic electrocardiographic changes indicative of cardiac involvement in a patient with AL amyloidosis: low-voltage and pseudo-anteroseptal infarction

effective in only 25% of patients. An important disadvantage of this approach is that it may take months before a clonal and clinical response becomes apparent. Especially patients with rapidly progressive disease may die due to progressive amyloid deposition before they have had the chance to respond. The addition of colchicine, which inhibits the induction of amyloid in mice and reduces formation of AA amyloid in familial Mediterranean fever, was of no benefit.[8,10] Therefore more aggressive therapies with a much faster effect on precursor-production have been investigated.



Figures 2A and B

SAP (Serum Amyloid P component) scans (24 hours after administration of SAP) of a patient with AL amyloidosis who had a partial clonal response to VAD (vincristine, doxorubicin, dexametahasone), HDM (high-dose melphalan), and ASCT (autologous stem cell transplantation) therapy and who had been followed for three years. Clinical response (disappearance of liver enlargement and normalisation of alkaline phosphatase levels) was reflected by serial SAP scintigraphy. A: Anterior views with intensive uptake in liver at start. After one year, liver uptake diminishes and becomes normal after two years. From two years the normal picture of blood pool activity in heart and major blood vessels and some degradation products in stomach and bladder is visible.

B: Posterior views at the same moments as A. Intensive uptake is present in the liver and also in the spleen at start. Splenic uptake decreases dramatically, although some uptake still remains at the end of follow-up.

In multiple myeloma, VAD (vincristine, doxorubicin, dexamethasone) may induce a quick clonal response in patients with previously untreated and refractory disease.[14,15] Whether VAD is useful in AL amyloidosis is not clear yet. Vincristine often has to be omitted because of the presence of peripheral or autonomic neuropathy and due to its potential cardiac toxicity doxorubicin cannot be used for a long period. In patients with cardiac amyloid involvement and ejection fraction less than 45%, the dose of doxorubicin should be adjusted or even completely omitted.

In our experience the clonal response to (V)AD used as single treatment is relatively short lived. Nevertheless, (V)AD might be a useful initial approach and this regimen is incorporated in treatment modalities for patients with AL amyloidosis younger than 65 years, as in the currently performed prospective HOVON 41 trial in patients under 66 years.

In the last decade intensive chemotherapy with high-dose melphalan (HDM, 200 mg/m²) followed by reinfusion of autologous stem cells (ASCT) has been applied in AL amyloidosis. Five studies reporting more than ten individually described patients with AL amyloidosis actually treated with HDM and ASCT have been published, with a total of 133 patients (*table 3*).[9,16-19] Recently, Skinner *et al.* published a study of melphalan and autologous stem cell transplantation in AL amyloidosis over an eight-year period (1994 to 2002), using several sequential protocols. Out of 701 consecutive patients with AL amyloidosis, 155 patients were actually treated with HDM and ASCT, 130 of whom had not been described before. Unfortunately, concerning clonal response, clinical response and treatment-related mortality, these 155 patients were not described separately but together with 122 patients who received an intermediate dose of melphalan (100-140 mg/m²).[20]

As expected, clonal and clinical responses are closely related. In the three studies in which the relation between both could be retrieved,[9,18,19] clinical response was seen in 28 of 40 patients (70%) who had a clonal response. The remaining 12 patients probably had stable clinical disease, because clinical worsening was not described in any of them. Clonal (complete or partial) response rates vary from 53 to 83%. The study by Skinner *et al.* showed 40% complete haematological response for the group of HDM and IDM treated patients together. Complete haematological response turned out to be positively associated with the dose of melphalan. Noteworthy, a significant survival advantage was noted for patients who achieved complete clonal response, compared with partial clonal response.[20] Clinical response rates vary from 42 to 83%, strongly depending on the time that passed after transplantation. Resolution of amyloid takes time and the rate of resolution of amyloid varies considerably among different individuals. Most responding patients show clinical response after three to six months (see *figure 2*), although later responses have been noticed (see *figure 3*). Importantly, a complete clonal response is not a prerequisite for clinical response and in patients with partial clonal response clinical improvement may still be noticed (see *figures 2* and *3*). On the other hand, a complete clonal response shows significantly higher clinical response compared with partial clonal response, 66% *vs* 30%[20] respectively. The equilibrium of formation and regression of amyloid deposits apparently moves towards regression. Noteworthy, the quality of life of responding patients improves, in contrast to patients responding to previously mentioned therapeutic modalities.
Treatment-related mortality (TRM) rates are high and vary between 13 and 43%. This is much higher than in MM, where rates below 5% are usually seen. This high TRM is

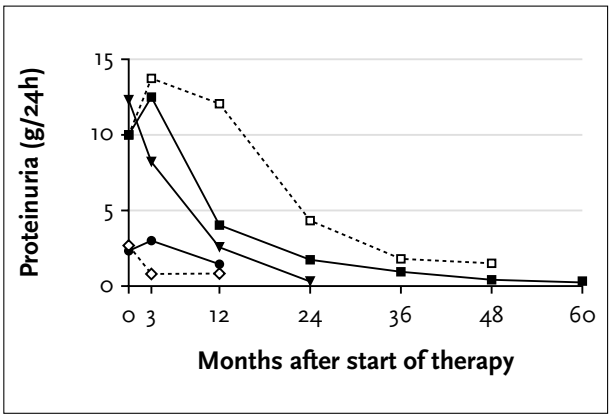


Figure 3
Monitoring of proteinuria in five patients with AL amyloidosis. All patients are responding to treatment with three courses of VAD (month 0-3) followed by HDM + ASCT (month 4-5). Notice the different pace of regression of proteinuria in time. All patients had partial clonal response.

Table 3
Five studies reporting ten or more selected patients with AL amyloidosis actually treated with HDM and ASCT

REFERENCE	YEAR	CENTRE	NUMBER OF PATIENTS	TRM	CLONAL RESPONSE	CLINICAL RESPONSE	CLINICAL RESPONSE (% OF CLONAL)
16	1998	Multicentre	40	15/40 (37%)	18/25 (72%)	14/25 (56%)	ND
17	1998	Multicentre	21	9/21 (43%)	ND	10/12 (83%)	ND
9	1998	Single centre	23	3/23 (13%)	13/20 (65%)	13/20 (65%)	10/13 (76%)
19	2002	Single centre	38	*	20/38 (53%)	16/38 (42%)	13/20 (65%)
18	2002	Single centre	11	2/11 (18%)	7/9 (78%)	6/9 (67%)	5/7 (71%)

HDM = high-dose melphalan (200 mg/m²), ASCT = autologous stem cell transplantation, TRM = treatment-related mortality (occurring in the first one to three months after HDM), ND = not described. Clonal and clinical responses were studied in the patients who survived the first three to four weeks after HDM.
**In this study both HDM and IDM (intermediate-dose melphalan) were reported, TRM was only mentioned for the whole group of patients (HDM and IDM together), and not for the patients treated with HDM only.*

caused by organ dysfunction in AL amyloidosis. The wide variation of TRM in the different studies is probably due to patient selection, experience, and the conditioning regimen used. Negative prognostic factors are (symptomatic) cardiac involvement and the number of vital organs involved. Especially patients with more than two vital organs affected have a high treatment-related mortality. Therefore careful patient selection for high-dose treatment is essential. Comenzo and Gertz published a risk-adaptive approach in which cardiac involvement and involvement of more than two vital organs were exclusion criteria for ASCT.[21] In the current Dutch study protocol (HOVON 41) and in the study by Skinner *et al.* clinical condition (measured by NYHA score for heart failure and WHO performance score), cardiac status and age are the most important factors in determining whether a patient is suitable for intensive treatment.[20]

Median follow-up in the five individually described studies is 25 months, and median survival has not been reached. Median survival of patients actually treated with HDM and ASCT in the study by Skinner *et al.* is 7.8 years, and five-year survival 61%.[20]
As Dispenzieri *et al.* pointed out, eligibility for stem cell transplantation in AL amyloidosis may be the most important favourable prognostic factor for survival.[22] Selection in this study was strict since Dispenzieri *et al.* retrospectively found only 18% of their patients eligible for HDM and reinfusion of autologous stem cells. In Groningen, 38% of patients were eligible for HDM followed by ASCT between 1985 to 2002.
Comparing survival of patients actually treated with HDM followed by ASCT with patients retrospectively eligible for this treatment showed a trend towards better survival in the patients actually treated.[18,20] No prospective study has been performed in which eligible patients treated with conventional therapy (oral melphalan/prednisolone) were compared with patients treated intensively (HDM followed by ASCT).

Since mortality in HDM followed by ASCT is high, intermediate-dose melphalan (IDM, 100 to 140 mg/m^2) followed by reinfusion of autologous stem cells has been applied. Indeed, treatment-related mortality turned out to be lower (15%) at a cost of lower response rates (25 to 30%)[21] and significantly lower survival of IDM and ASCT *vs* HDM and ASCT: median survival 2.9 and 7.8 years respectively, and five-year survival respectively 41 and 61%.[20,23] Other chemotherapeutic regimes are anecdotal, such as low-dose intravenous melphalan (25 mg/m^2, once every four weeks, four to six times),[24] continuous oral low-dose melphalan (4 mg/day, five days a week),[25] or oral cyclophosphamide. The application of allogeneic bone marrow transplantation is also anecdotal.[26]

Thalidomide and other new antimyeloma drugs
In MM thalidomide has shown to be well tolerated and highly effective in patients resistant to alkylating therapy.[27] The exact antitumour mechanism of thalidomide is still elusive, although antiangiogenesis and immunological effects are thought to be important factors. The limited experience so far with thalidomide in AL amyloidosis has shown a high intolerability, especially oedema, neuropathy, constipation, syncope due to bradycardia, and thromboembolic complications. Therefore, it was withdrawn in 25 to 70% of the patients, and the maximum tolerable dose was often low (below 200 to 300 mg).[28-30]
Recently phase II studies with new, potent antimyeloma drugs in patients with MM have been published. The thalidomide analogue (CDC-5013) and the proteasome inhibitor bortezomib (formerly called PS-341) showed promising clinical activity with acceptable toxicity profile in heavily pretreated patients.[31,32] Bortezomib is a novel dipeptic boronic acid, which downregulates *in vitro* the NF-kappaB pathway. *In vitro*, bortezomib has shown potent activity and also enhanced the sensitivity of cancer cells to traditional chemotherapy.[32] Both drugs might be promising in AL amyloidosis in the near future.

Prohibiting formation or stimulating degradation of amyloid fibrils
To date, there are no drugs available for degrading amyloid depots in tissue, although three drugs are under investigation.
Two trials have been performed with 4'-iodo-4'-deoxydoxorubicin (IDOX), a halogenated anthracycline derivative with significantly reduced cardiac toxicity compared with currently used anthracyclines.[33,34] IDOX can bind to amyloid fibrils, leading to catabolism of amyloid deposits, and has shown to have some effect in soft tissue involvement. The dosing schedule is still under investigation.
Pepys *et al.* are currently investigating R-1-[6-[*R*-2-carboxypyrrolidin-1-yl]-6-oxohexanoyl]pyrrolidine-2-carboxylic acid (CPHPC).[35] CPHPC blocks the binding sites of serum amyloid P component (SAP) and also cross-links pairs of pentameric SAP molecules. The SAP-CPHPC molecule clearance is high, leading to depletion of SAP from the circulation, and in animal studies reduction of amyloid deposits has been shown.
The third drug, currently being investigated in a multinational phase II/III trial, is sodium-1,3-propane-disulfonate (Fibrillex, or NC-503). In animal studies this low-molecular drug has shown an inhibitory effect on deposition of AA amyloid. Amyloidogenic proteins bind to glycosaminoglycans in tissue, which enhances the process of polymerisation and deposition of amyloid in the extracellular matrix. Fibrillex is a glycosaminoglycan-mimetic drug that competitively binds to the precursor protein SAA, thereby prohibiting the binding to glycosaminoglycans in

tissue.[36] In AL amyloidosis, however, the variety of different precursor proteins (both kappa and lambda) is wide. Therefore the chance is small that one glycosaminoglycan-mimetic drug can be developed that binds to all possible precursor proteins of AL amyloid.

CONCLUSION

To date, treatment with high-dose melphalan and autologous stem cell transplantation in patients with AL amyloidosis seems to be superior to other forms of (chemo)therapy and therefore should be considered in any patient with AL amyloidosis. Clonal and clinical response rates are high, and survival is prolonged. Due to high treatment-related mortality this therapy is only optional for a subgroup of patients without advanced vital organ involvement, especially of the heart, and good performance status. To increase the number of patients eligible for this treatment, early diagnosis is extremely important.

Patients not eligible for HDM and ASCT could benefit from IDM and ASCT at the cost of lower clonal and clinical response rates and significantly lower survival. Other chemotherapy regimens, such as orally administered melphalan and prednisolone, seem to be less effective but should be tried in patients not eligible for HDM or IDM. So far the role of thalidomide in AL amyloidosis seems to be limited due to high intolerability. New drugs, such as bortezomib and the thalidomide analogues now tested in myeloma, and the development of drugs that are able to resolve amyloid deposits may become important in the near future. Given the low incidence of AL amyloidosis and the potential difficulties in treating patients, treatment should be restricted to or in close collaboration with centres experienced in the management of this disease.

NOTE

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ORIGINAL ARTICLE

Diagnosis and treatment of levothyroxine pseudomalabsorption

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ABSTRACT

Many causes of malabsorption of levothyroxine in patients with hypothyroidism have been thoroughly described in literature. Pseudomalabsorption, poor compliance of the patient with the therapy regime, is the most common cause of failure of levothyroxine therapy. Pseudomalabsorption is characterised by a deficient diagnostic process, patient denial and difficulties in treatment. The present article provides guidelines in diagnosing and treating pseudomalabsorption in hypothyroidism.

INTRODUCTION

Thyroid diseases are common in the endocrinology clinic.[1] Well-documented clinical trials on therapeutic options are available in the case of hypothyroidism.[2,3] Prevalence of hypothyroidism amongst adults is 15.9 out of 1000 persons.[1] As a result of dysfunction of the thyroid gland, the free thyroxine (fT_4) is characteristically low, sometimes even reduced to undetectable concentrations. The thyroid-stimulating hormone (TSH) serum value is elevated as a result of the regulatory negative feedback mechanism. Patients with hypothyroidism are supplemented with synthetic thyroxine hormone (i.e. levothyroxine, LT_4) in oral doses to achieve physiological fT_4 serum levels. The mean treatment dosage LT_4 is 1.6 µg/kg bodyweight a day.[4] The results with this dosage are adequate and reproducible. When, however, large amounts of LT_4 are needed for hypothyroidism treatment, the cause should be investigated by the clinician. Many causes of LT_4 malabsorption are known and discussed in literature (*table 1* on the next page). Common causes are gastrointestinal diseases, liver diseases, pancreatic diseases, certain gastrointestinal surgical procedures, drugs and dietary interactions, heart disease or pregnancy.[5-30] By far the most common cause of malabsorption is, however, poor or noncompliance with oral LT_4 treatment by the patient.[31,32] Even with this knowledge, the above-mentioned possible causes should initially be considered.

The aim of the article is to provide guidelines for the physician for the investigation of malabsorption of LT_4. Probable causes and their specific investigations are discussed. In addition, possible techniques to detect pseudomalabsorption are proposed. For this purpose a typical case of pseudomalabsorption is presented.

CASE PRESENTATION

A 33-year-old female patient presented to the outpatient endocrinology clinic with the typical symptoms of hyperthyroidism (i.e. agitation, weight loss, increased sense of hunger and diarrhoea). Initial laboratory blood investigations showed an increased fT_4 serum concentration (108.3 pmol/l) and TSH concentrations below detection level (<0.05 mE/l) (*figure 1*). Ultrasonography of the thyroid gland showed inhomogeneous lobes without cysts or nodules. The right lobe measured 2.4 x 2.1 x 4 and the left lobe 1.5 x 2.0 x 3.0 cm. Laboratory tests for thyroid-stimulating immunoglobulins were positive and the diagnosis of Graves hyperthyroidism was made. Treatment was started with thiamazole and propranolol in daily doses. After four months of treatment normalisation of fT_4 and

Table 1
Biological causes of levothyroxine malabsorption

HYPOTHYROIDISM	
Gastrointestinal diseases	Coeliac disease[38]
	Lactose intolerance[39]
	Vitamin B_{12} deficiency[40]
	Intestinal infections (*Giardia lamblia*)[41]
Liver diseases	Cirrhosis
	Obstructive liver disease[42]
Pancreatic diseases	Pancreatic insufficiency[5,6]
Previous gastrointestinal surgery	Jejunostomy[9]
	Jejunoileal bypass[7,8]
	Short bowel syndrome[10]
Medication interference	Cholestyramine[14]
	Colestipol[15]
	Aluminum hydroxide-containing antacids[16]
	Ferrous sulphate[17,18]
	Sucralphate[19]
	Propranolol[20]
	Laxatives[21]
	Calcium carbonate[22,23]
	Lovastatin[24]
	Bile acid sequestrants[14]
	Activated charcoal[43]
	Anion exchange resins[5]
	Phenytoin[26]
	Phenobarbital[26]
	Carbamazepine[27]
	Rifampin[28]
	Amiodarone[29]
	Oestrogen therapy[11]
Dietary interference	Walnuts[12]
	Soybean[13]
	Prunes[12]
	Herbal remedies[30]
Heart disease	Congestive heart failure[5]
Pregnancy[11]	

Biological causes of levothyroxine malabsorption as discussed in medical literature.

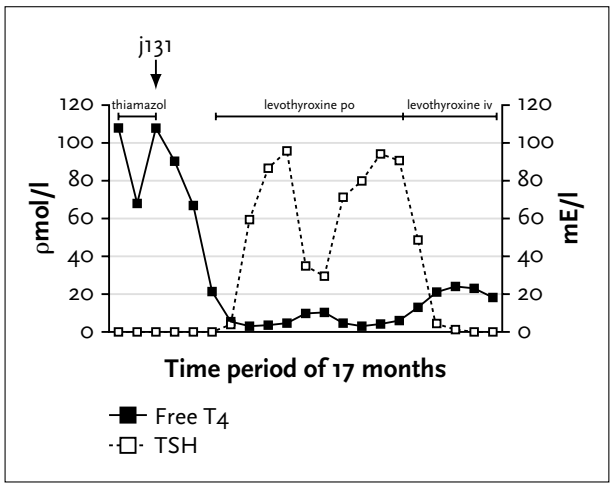

Figure 1
Serum concentrations free T4 and TSH

TSH to the physiological level had still not been reached. The decision was made to inhibit thyroid functioning with radioactive iodine. Scintigraphic visualisation showed a relatively enlarged thyroid gland with intense homogeneous activity and a 24-hour radioactive iodine 131 (J131) uptake of 74%. The patient was given 15 mCi radioactive iodine orally. Concordantly, the patient developed hypothyroidism with low fT_4 and high TSH serum concentrations concomitant with the characteristics of attenuated thyroid gland functioning: weight gain, fatigue, myxoedema, muscle cramps and constipation. The patient was treated with LT_4 orally for the acquired endocrine dysregulation. Normalisation of the fT_4 and TSH concentrations was not reached during treatment with oral LT_4. Even high dosages of 400 μg per day were not successful. Malabsorption of LT_4 through adjunctive medicine or supplement use was excluded. Gastrointestinal, liver or pancreas diseases were excluded through laboratory investigations. Additionally, the patient had not had previous gastrointestinal surgery. Congestive heart failure and pregnancy were not present either. Consequently, the patient was admitted to hospital to investigate the cause of the unsuccessful treatment more thoroughly. Intravenous LT_4 treatment was started to treat the symptoms of hypothyroidism and, additionally, investigate the option of an autoimmune action against thyroxine hormone. Immunological investigations targeted on intestinal malabsorption, such as lactose intolerance or lactase deficiency, were negative. Antibodies against gliadine and endomysium were not present. Intestinal germs as *Giardia lamblia* were excluded. Normal fT_4 and TSH serum levels were reached with 200 μg LT_4 intravenously within 12 days. Moreover, the symptoms diminished within this period. The physical state of the patient improved greatly and oral LT_4 treatment was tried once more. However, in one week the endocrinological state of hypothyroidism was reached again. The possibility of malabsorption by defective compliance (i.e. pseudo-malabsorption) was the most probable cause at this stage. To prove pseudomalabsorption an LT_4 absorption test was performed. After an overnight fast the patient was not allowed to ingest anything except fluids during the duration of the test. An oral dose of 1000 μg LT_4 was given under the auspices of the physician. The patient was observed by a trained nurse throughout the test. Blood samples were obtained prior to, and two, four and six hours following

the bolus ingestion to investigate total T_4 (TT_4), fT_4 and TSH serum levels. *Figure 2* shows the results of the absorption test as performed in the presented case. The patient started with laboratory signs coinciding with hypothyroidism. Immediate fT_4 serum increase is seen following LT_4 ingestion, with the maximum serum level within the first 120 minutes, known to be a normal time interval.[33] The results therefore showed a normal absorption of LT_4 by the small intestines, and malabsorption was excluded; pseudomalabsorption was proven.

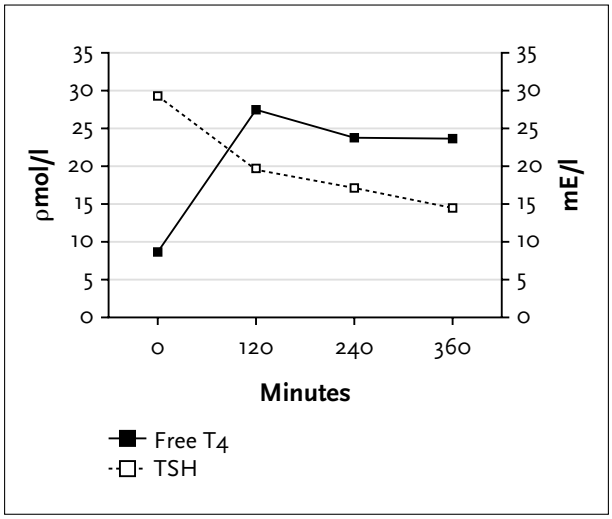

Figure 2
Serum free T4 and TSH response to bolus levothyroxine administration

DISCUSSION

A case such as this is rarely seen in the endocrinology clinic, although it is typical in its essence. The clinician was challenged with a situation of hyperthyroidism, which did not respond to treatment. The choice was made to improve the condition of the patient by treatment with radioactive iodine, after which hypothyroidism developed. This endocrinological dysregulation did not improve despite much effort from the clinician and high dosages of oral LT_4.

Pseudomalabsorption is part of the differential diagnosis of every doctor, although the great disadvantages of hypothyroidism for the patient (such as fatigue, constipation, weight gain up to 27 kg in 12 months) would seem reason enough for therapy compliance. However, from the Munchhausen syndrome it is known that patients are capable of low compliance and exposure to serious complications of unnecessary medical and surgical procedures for doubtful reasons.[34] Before the diagnosis pseudomalabsorption can be made, all possible causes for malabsorption should be investigated (*table 2*). The combination of clinical presentation, thorough history taking, exploration of diet and drugs and primary laboratory investigations is able to exclude most known causes of LT_4 malabsorption. For the most common cause, pseudomalabsorption, an LT_4 absorption test is diagnostic. The absorption test is performed with 1000 to 2000 µg LT_4 orally with control of proper ingestion and possible surreptitious regurgitation.[4,33,35] LT_4 uptake is greater when fasting, so patients are kept fasting in advance of the absorption test.[4] Normally 70 to 100% of the administered dose is absorbed within the gastrointestinal tract, with maximal serum levels reached within two to four hours following ingestion.[4,33,35] It should be noted that the intestinal uptake of LT_4 is variable among euthyroid subjects.[4,33,35] A TT_4 distribution volume of 13 to 17% and a maximum TT_4 serum level rise of 116 nmol/l (1 nmol T_4 is about 750 ng) reveals nearly 100% LT_4 absorption in our patient.[4,35] However, even the normalisation of fT_4 and TSH serum levels following LT_4 ingestion prove the absence of an intrinsic absorption defect. An absorption test as performed is able to distinguish between pseudo and real malabsorption, even between different forms of intrinsic absorption defects.[33,36] From our results, we concluded that the hypothyroid state of our patient could be explained by pseudomalabsorption of LT_4, i.e. poor treatment compliance.

Treatment of LT_4 pseudomalabsorption is hampered by the general poor compliance and absence of recognition of the patient. The poor compliance is often due to psychiatric

Table 2
Diagnostic process of pseudomalabsorption

HYPOTHYROIDISM	
Exclude	Gastrointestinal diseases
	Liver diseases
	Pancreatic diseases
	Previous gastrointestinal surgery
	Medication interference
	Dietary interference
	Intraluminal germs
	Congestive heart failure
	Pregnancy
Test pseudomalabsorption	Levothyroxine absorption test
	Intravenous levothyroxine treatment

In the case of untreatable hypothyroidism, unresponsive to high-dose levothyroxine treatment, the possibility of a biological cause should be explored. If no indications for biological causes are found, the possibility of poor patient compliance should come forward, and could be tested through a levothyroxine absorption test or intravenous levothyroxine treatment.

disorders of a depressive nature, which are not uncommon in severe hypothyroidism, although few patients exhibit true psychopathology.[4,35,37] Treatment, therefore, needs unusual measures. Psychiatrists recommend that patients presenting the psychopathological features of a Munchhausen syndrome or factitious disorder should be observed conservatively.[34] Confronting the patient with the doubts of the clinician about the level of compliance could mark and mutilate the patient for life, without improvement in the treatment. Subtle handling of the patient is required. On the other hand, the state of hypothyroidism should be improved. Several treatment strategies are possible. Parenteral infusion of LT_4 has shown to be useful in (pseudo)malabsorption.[36] Supervised oral LT_4 ingestion is a less invasive alternative. Patients are prone to drop out of both treatment regimes. Informing the patient about the effects of poor compliance does improve the five-year compliance follow-up in some patients though.[4]
Our first objective was to diminish the disadvantages of hypothyroidism by intravenous LT_4 treatment. We prepared the patient for a restart of oral LT_4 intake within the process of feeling better physically. When this proved to be unsuccessful an absorption test was performed. The dose given was in line with a one-week dose. The patient was therefore subscribed one dose of LT_4 weekly. The fT_4 and TSH serum levels normalised and the patient continued to do well during follow-up.
In conclusion, pseudomalabsorption is characterised by a troubled diagnosis process, absence of recognition of the patient and difficulty in treatment. The present article provides guidelines in diagnosing and treating LT_4 pseudomalabsorption in hypothyroidism.

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Lips, et al. Pseudomalabsorption of levothyroxine.

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Two patients with recurrent fever and wine red discolouration of the eyelids

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CASE REPORT

A 65-year-old woman was seen in our outpatient clinic because of recurrent febrile attacks since the age of six years. The medical history revealed appendectomy and diagnostic laparotomy during childhood because of unexplained fever and abdominal pain. The attacks are characterised by spiking fever up to 40°C twice a day, conjunctivitis, abdominal pain, erythematous skin lesions, myalgia, arthralgia especially of hands and legs and an intense acute phase response. During the last episode the erythrocyte sedimentation rate was 100 mm/h, C-reactive protein serum concentration 219 mg/l and leucocyte count 34.2 x 10^9/l. The attacks generally last two to three weeks and recur two to five times a year. In between two episodes there are no symptoms. Extended clinical observation, CT scanning of the thorax and abdomen, indium 111-IgG scanning, multiple cultures of body fluids and microscopic examination and cultures of bone marrow did not reveal a diagnosis. Treatment with prednisone (20 mg/d orally) and NSAIDs during attacks alleviated symptoms but had no influence on the duration and recurrence rate of the attacks. The family history revealed that father, brother, brother's daughter and her own daughter experienced similar attacks. Over the past two decades she developed a progressive, slightly elevated wine red discolouration of the skin surrounding the eyes and the eyelids (*figure 1* left panel), which became more prominent and itched during attacks.

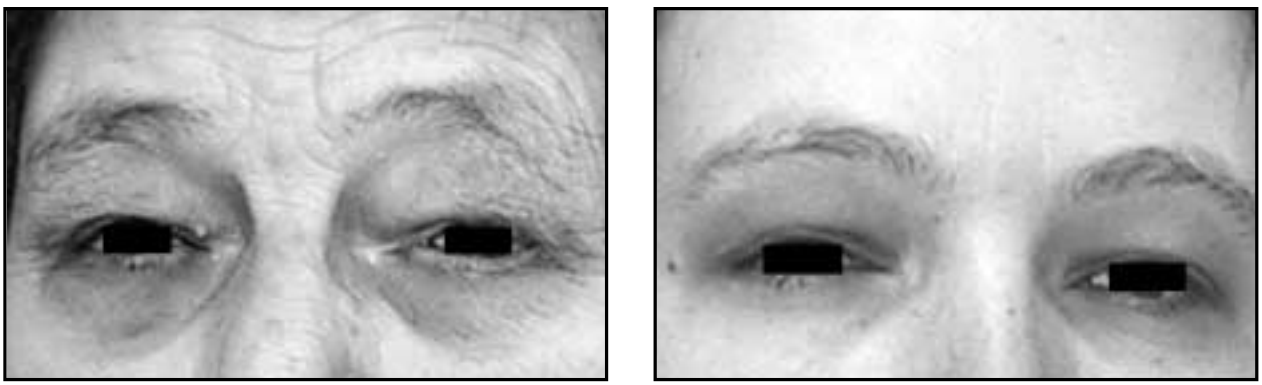

Figure 1
Wine red discolouration of the eyelids and the skin surrounding the eyes (left panel mother, right panel daughter)

A colour version of this photo quiz can be found on our website www.njmonline.nl.

Her now 35-year-old daughter has had similar attacks since the age of four, characterised by spiking fever up to 40°C, myalgia, arthralgia in hands and legs, migrating guirlande-like erythematous cutaneous lesions on the trunk and an acute phase response. These attacks last for two to three weeks and recur two to three times a year. During the most recent visit to our outpatient clinic we observed a wine red discolouration of the eyelids and the skin surrounding the eyes resembling that of her mother (*figure 1*, right panel).

WHAT IS YOUR DIFFERENTIAL DIAGNOSIS?

See page 139 for the answer to this photo quiz.

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REVIEW

Diagnostic and therapeutic approach of systemic amyloidosis

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ABSTRACT

Amyloidosis is a group of diseases, all characterised by deposition of protein fibrils with a β-sheet structure. This structure generates affinity of amyloid for Congo red dye and is resistant to proteolysis. Three types of systemic amyloidosis are important for the clinician: 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 considerably among the three types and the choice of treatment differs completely.

A stepwise approach in diagnosis and therapy is presented. When amyloidosis is suspected the first step is histological proof of amyloid and the second is proof of systemic involvement. The next two steps are determination of the type of amyloid followed by detection of the precursor protein. The fifth step is a thoughtful clinical evaluation, necessary for assessment of prognosis and therapy. Subsequently, the choice of therapy is based on the 'precursor-product' concept. In the final step, the effects of therapy on the underlying disease as well as on the amyloidosis are assessed during follow-up. In this evaluation serum amyloid P component (SAP) scintigraphy helps to show organ involvement and therapy response.

INTRODUCTION

Amyloidosis is a group of diseases all characterised by deposition of proteinaceous fibrils with a molecular β-sheet structure.[1] This structure of the fibrils is responsible for their insolubility, resistance to proteolysis and binding affinity for Congo red dye and the consequent green birefringence with polarised light. Amyloid fibrils are derived from different protein precursors. Extracellular deposition of amyloid fibrils in organs and tissues results in loss of organ function and may cause prominent swelling of the affected organ or tissue. Deposition of amyloid can be localised (restricted to one organ or site of the body) or systemic (in various organs and tissues throughout the body). The various clinical pictures of systemic amyloidosis are related to the type of precursor protein involved.[1,2] Terms such as primary and secondary amyloidosis have become obsolete, because all types of amyloid are secondary to the production of a specific precursor. Therefore the old nomenclature has been replaced by a new one based on the protein precursor.[2] In this article the clinician will find a stepwise approach to diagnosis, clinical evaluation and background of therapy in patients with suspected systemic amyloidosis. Readers who want to know more about clinical aspects and molecular mechanisms of the systemic amyloidoses are referred to the review articles of Falk *et al*[1] and Merlini and Belotti.[3]

CLASSIFICATION

Although localised deposition of amyloid plays an important role in the development of widespread serious diseases such as Alzheimer's disease (β-protein in the plaques) and diabetes mellitus type II (amylin in the islands of Langerhans), this article focuses on the systemic types of amyloidosis. There are four major types.[1,3]

AA amyloidosis
This type is caused by longstanding inflammation. Serum amyloid A protein (SAA), an acute phase reactant, is the precursor protein. Renal manifestations, such as proteinuria (progressing to nephrotic syndrome) and loss of renal function (progressing to renal failure), are observed very frequently (about 90% of cases). Less frequent manifestations are autonomic neuropathy, hepatomegaly and cardiomyopathy.

AL amyloidosis
AL amyloidosis is caused by a plasma cell dyscrasia. Lambda or kappa immunoglobulin light chain is the precursor protein of this type. Clinical manifestations are very diverse, such as cardiomyopathy, hepatomegaly, splenomegaly, nephrotic syndrome, renal failure, orthostatic hypotension, diarrhoea, peripheral and autonomic neuropathy, arthropathy, carpal tunnel syndrome (CTS) and glossomegaly. The diversity of manifestations (and their combinations) depends on the severity of deposition in the various organs and tissues.

ATTR amyloidosis
Various autosomal dominant hereditary point mutations of the precursor protein transthyretin (TTR) cause this type. Transthyretin, formerly called prealbumin because of its electrophoretic profile, is an acronym of a transport protein of thyroid hormone and retinol-binding protein. More than 80 of these mutations have been described. Prominent clinical manifestations are (familial) peripheral and autonomic neuropathy, but cardiomyopathy, renal failure and eye involvement (vitreous opacities) are also often observed. Severe cardiomyopathy can be the presenting sign in some of the TTR mutations. In very old age, normal ('wild-type') TTR can also behave as precursor protein. This so-called senile systemic amyloidosis is characterised by a slowly progressive cardiomyopathy.

Aβ2M amyloidosis
This type is caused by renal failure and longstanding (i.e. at least 5 to 10 years) dialysis with decreased clearance of beta-2-microglobulin (β2M). β2M is the precursor protein of this type. Clinical manifestations are arthropathy, such as tenosynovitis, shoulder pain, CTS, periarticular cysts, pathological fractures and destructive spondyloarthropathy. Synovial tissue biopsy is the method to detect amyloid. Kidney transplantation stops the disease.[4,5]
Aβ2M amyloidosis is a disabling disease that should be recognised and treated. This article, however, describes only the first three types (AA, AL and ATTR) of systemic amyloidosis because these types are often unexpected, difficult to diagnose, with variable involvement of many different organs and tissues and often pose the problem of finding the most appropriate therapy.

PROOF OF AMYLOID

The first step is to detect amyloid. The diagnosis of amyloid is based on proof of its presence in tissue. This can be shown in a positive Congo red stained tissue specimen with the characteristic apple-green birefringence in polarised light (see *figure 1*). The abdominal subcutaneous fat aspiration is the most elegant and least inconvenient method for this purpose, with a sensitivity ranging between 54%[6] and 82%[7,8] and a specificity of 100%.

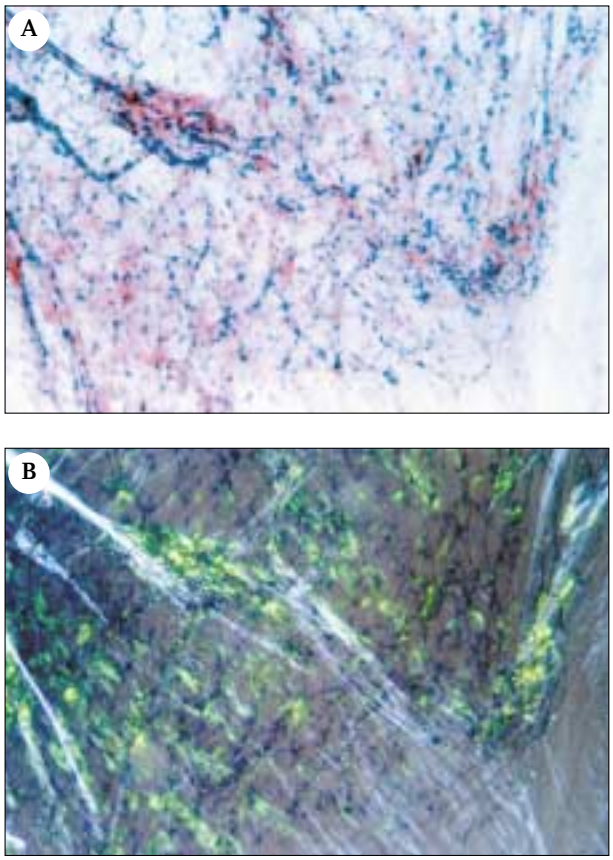

Figure 1A and B
Example of an abdominal subcutaneous fat aspirate, stained with Congo red, magnification 30x.
A: When viewed in normal light, amyloid is stained red.
B: The same specimen viewed in polarised light: amyloid shows apple-green birefringence.

These figures are similar to those of the well-known rectum biopsy.[8] If the primary biopsy site (fat or rectum) is negative for amyloid and there is strong suspicion of amyloidosis, a biopsy of the other tissue is useful to increase the chance of detecting amyloid. A bone marrow biopsy can also be used, but has a disappointingly low sensitivity of 50 to 60%.[8] When all biopsies are negative but a strong suspicion of amyloidosis still exists, a biopsy of the affected organ or tissue is indicated.[1,9]

SYSTEMIC DEPOSITION

Amyloid deposition can be local or systemic. Therefore the second step is to check for systemic deposition. Some sites are exclusively involved in systemic amyloidosis, such as kidneys, liver, nerves, abdominal fat and spleen. If such a site is positive for amyloid, systemic involvement is established. Localised amyloid can often be found in other sites of the body, including the eyelid, cardiac atria, larynx, ureter and skin. In these cases amyloid must be undetectable elsewhere in the body to confirm localised amyloidosis. Most other sites (bone marrow, heart, bowel, lung, joint, etc.) are nearly always involved in systemic amyloidosis. In this situation it is recommended to demonstrate 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 clinical involvement (such as nephrotic syndrome, hepatomegaly, macroglossia, or cardiomyopathy) at the other site.[10]

TYPE OF AMYLOID

After verification of presence of systemic amyloid, the third step is determination of the type of amyloid. In many cases the type of amyloid can be assessed with high probability from the medical history and clinical picture. Amyloidosis in a patient with longstanding rheumatoid arthritis and nephrotic syndrome is almost certainly the AA type. Someone with polyneuropathy who belongs to a family with hereditary amyloidosis probably has ATTR amyloidosis. And in a patient with characteristic shoulder pads and glossomegaly it is hard to imagine something other than AL amyloidosis. Nevertheless, even in these patients with strong clinical evidence for a particular type of amyloid, more solid confirmation of the specific type of amyloid should be determined. The clinical consequences of incorrect typing of amyloid can be considerable: prognosis and therapy of the three major types of systemic amyloidosis are completely different.
Immunohistochemistry of a biopsy is helpful to characterise the type of amyloid by using specific antibodies (see *figures 2* and *3*). In AA amyloidosis this technique is sufficient, provided sensitive and specific monoclonal antibodies are used, such as mc1[10] and Reu.86.2.[11,12]

However, in ATTR amyloidosis and especially in AL amyloidosis this method is less reliable than in AA amyloidosis. This is caused by heterogeneity of amyloid deposits, loss of epitopes in the fibril structure, lower sensitivity and specificity of (polyclonal) antibodies and nonspecific adherence of immunoglobulins to amyloid deposits or the background.[10] Lack of a positive family history does not exclude ATTR amyloidosis as shown by a considerable number of 'sporadic' cases that have been described.[13] Therefore presence of a TTR mutation (by DNA analysis) must be established in all cases of ATTR amyloidosis. The only exclusion for this requirement is old age (>80 years) and a typical clinical picture of senile systemic amyloidosis (i.e. slowly progressive cardiomyopathy). In patients with AL amyloidosis, a monoclonal plasma cell dyscrasia with overproduction of lambda or kappa light chain must 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 the free light chain assay). However, a monoclonal gammopathy of undetermined significance (MGUS) is frequently present in healthy older persons,

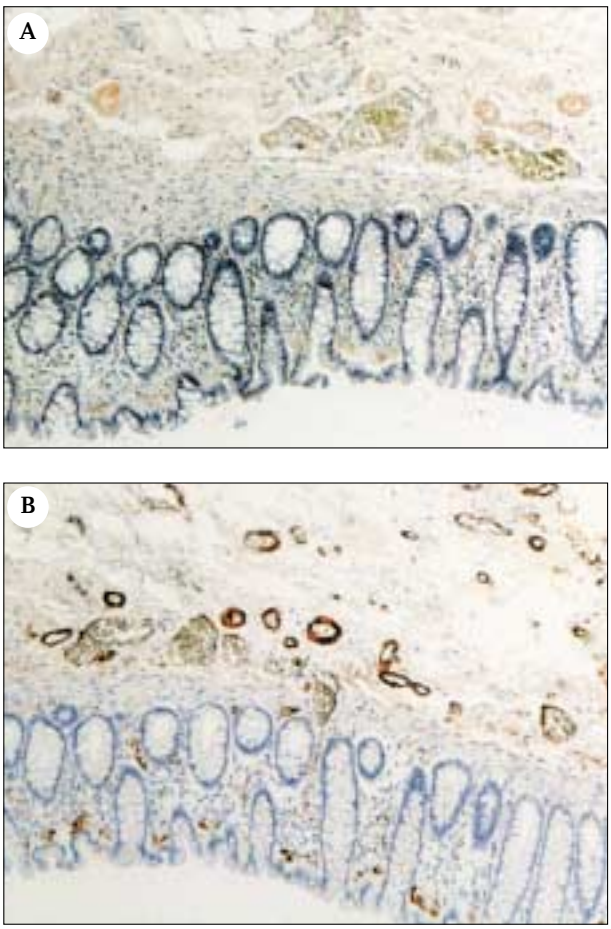

Figure 2A and B
Rectum biopsy of a patient with AA amyloidosis, magnification 30x. Small deposits of amyloid can be seen in the epithelial border and in the submucosa in the walls of blood vessels.
A: Amyloid is red in the Congo red stain.
B: Amyloid is brown in the immunoperoxidase stain with monoclonal antibodies against SAA (Reu.86.2).

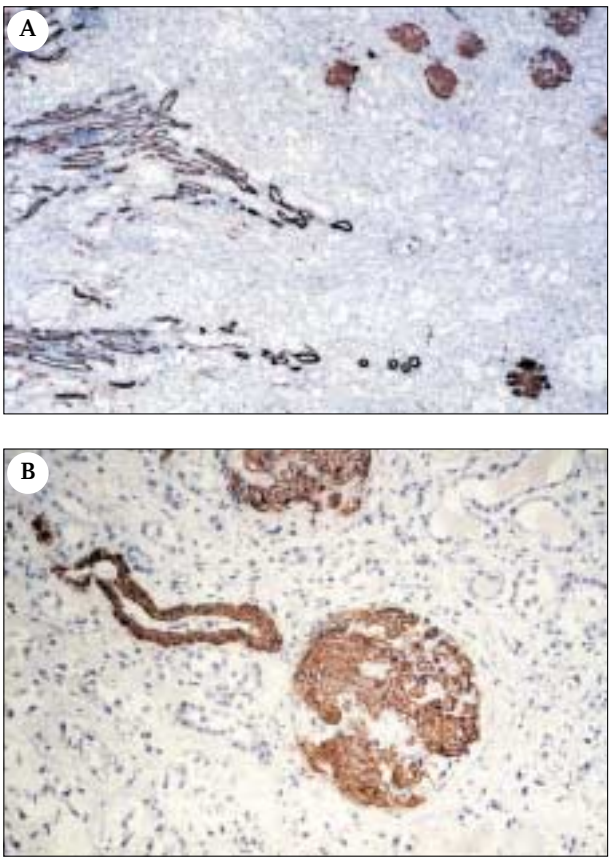

Figure 3A and B
Kidney tissue of a patient with AA amyloidosis. Amyloid is brown in the immunoperoxidase stain with monoclonal antibodies against SAA (Reu.86.2).
A: Overview with glomeruli and vasa recta, magnification 10x.
B: Detail, glomerulus, magnification 40x.

ranging from 2% in persons of 50 to 3% in persons of 70 years.[14] Thus detection of MGUS does not exclude other types than AL amyloidosis. It is important to notice that the clinical picture of ATTR amyloidosis and AL amyloidosis can sometimes be similar, such as in cases with polyneuropathy, autonomic neuropathy, cardiomyopathy and carpal tunnel syndrome. In 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 AL amyloidosis can be diagnosed.[13]

PRECURSOR PROTEIN

After establishing the type of amyloid it is time for the fourth step, i.e. to look for a precursor protein in the blood. Detection of a precursor protein and measuring its serum concentration is important for therapy. In AA amyloidosis the precursor protein is SAA, an acute phase reactant. The behaviour of SAA during inflammation is comparable with C-reactive protein (CRP), a protein that can be assessed in routine clinical practice. In ATTR amyloidosis the precursor protein is a mutated TTR. This can be detected by isoelectric focusing.[1] In AL amyloidosis a recently described assay shows the presence of free lambda and kappa precursor proteins in blood using specific antibodies raised against normally hidden epitopes in the complete immunoglobulin.[15]

CLINICAL EVALUATION

The fifth step is to obtain a reliable understanding 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 about the 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 helpful. 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), 24-hour Holter registration (conduction, rhythm and heart rate variability) and a MUGA scan (ejection fraction). The kidneys can be examined with serum albumin, creatinine clearance, urine sediment and proteinuria, whereas serum albumin, liver enzymes such as alkaline phosphatase, bilirubin, coagulation tests and cholinesterase can be used to examine the liver. 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 are ways of evaluating autonomic neuropathy.[16,17] Electromyography can be used to assess peripheral neuropathy. Abdominal ultrasound may be helpful to evaluate size and echogenecity of liver, spleen and kidneys. Not all of the examinations mentioned above need to be employed 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.
Serum amyloid P component (SAP) scintigraphy is a technique that has been developed in London by Pepys and Hawkins for specific evaluation of amyloidosis.[18,19] All amyloid deposits contain SAP, a glycoprotein that belongs to the pentraxin family and binds in a calcium-dependent way to all amyloid deposits independently of the protein of origin. The ^{123}I-labelled SAP scan can show specific uptake in organs such as liver, spleen, kidneys,

adrenals, bone marrow and joints (see *figure 4* for some examples). However, myocardium does not show specific uptake, probably because of the combination of high background activity of tracer still present in the blood pool and decreased permeability in cardiac tissue of this tracer with a high molecular weight.[18] Measurement of SAP retention after 24 or 48 hours combined with the intensity of organ uptake on images provides a quantitative estimate of amyloid load in an individual patient that might be used to monitor effect of therapy in this patient.[19] The technique is only available in a few centres.

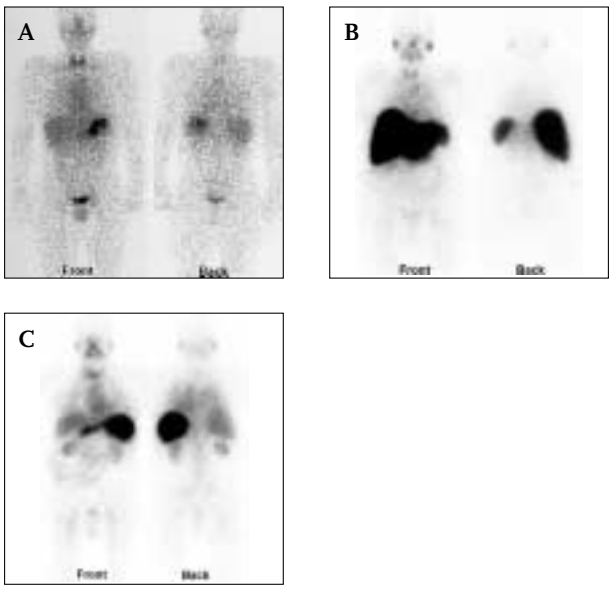

Figure 4A, B and C
SAP (Serum Amyloid P component) scintigraphy 24 hours after intravenous injection of ^{123}I-SAP, total body uptake front (left images) and back (right images).
A: Healthy control with minor nonspecific uptake (radioactive degradation products including free iodine) in stomach, bladder and minimal uptake in the (blocked) thyroid.
B: Intense uptake in liver and spleen in a patient with AL amyloidosis.
C: Uptake in spleen and kidney in a patient with AA amyloidosis.

PROGNOSIS

The last step before determining therapy is assessment of prognosis. Generally prognosis is poor if the disease is untreated. The prognosis depends upon the type of amyloid, severity of amyloid deposition, number of vital organs affected, presence of symptomatic cardiomyopathy, severity of the underlying disease and response to therapy of the underlying disease. Patients with untreated AL amyloidosis have the worst prognosis, with a median survival of less than one year.[1,9] Median survival in AL amyloidosis in case of symptomatic cardiomyopathy is four to six months, with kidney involvement about two years and with CTS more than three to four years. Patients with AA amyloidosis have a median survival of two to four years.[1,9] However, survival depends greatly on the activity of the underlying inflammation.[20] Patients with ATTR amyloidosis may survive up to 10 to 15 years.[1]

THERAPY

The current basis of therapy is the so-called 'precursor-product' concept.[21] The central idea of this concept is that further growth of amyloid deposits will cease when the supply of necessary precursor proteins is put to a stop. Therefore, in AA amyloidosis the 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 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 10 mg/l) can be difficult, but should be attempted.[20] To achieve this goal, cytostatic drugs can be used (such as methotrexate, azathioprine, cyclophosphamide, or chlorambucil), but also anti-TNF (tumour necrosis factor) drugs (such as infliximab, adalimumab and etanercept). In patients with TRAPS (TNF-receptor-associated periodic syndrome) etanercept (acting as a soluble TNF receptor) seems to be a rational treatment because of the abnormal function of the mutated TNF receptor.[22] The interleukin-1-receptor antagonist anakinra may be highly effective in cryopyrin-related diseases such as familial cold urticaria and Muckle-Wells syndrome.[23] 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.[24] Dimethylsulphoxide (DMSO) was first thought to dissolve amyloid fibrils, but turned out to be an anti-inflammatory agent.[25] The antiamyloid effect in AA amyloidosis appeared to be mediated by lowering SAA serum levels.[21,25,26]

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.[27,28] In patients with hereditary ATTR amyloidosis liver transplantation is nowadays the only way to remove the source of 99% of the mutated TTR in the blood.[29]

SUPPORTIVE TREATMENT

Beside treatment aimed at the underlying disease, it is necessary to give supportive treatment for loss of organ function caused by amyloid deposition. In cardiac involvement the clinician should be extremely careful when using digoxin and calcium-channel blockers (their affinity to amyloid in the heart may enhance toxicity) and with cisapride for bowel motility problems (because of the risk of 'torsade des pointes'). Amyloid involvement of the heart primarily leads to right-sided heart failure; therefore the clinician should be careful with volume depletion (the problem is more an inflow than an outflow problem). Patients with symptomatic bradycardia may need implantation of a pacemaker. Nephrotic syndrome can be treated with salt restriction, careful use of diuretics and angiotensin-converting enzyme (ACE) inhibitors or nonsteroidal anti-inflammatory drugs. Orthostatic hypotension is difficult to treat. Fludrocortisone should be tried first and sometimes erythropoietin may also be helpful to treat this condition. Amitryptilline can be used for neuropathic pain; however, it should be used with caution (because of its possible effects on blood pressure and rhythm) in patients with cardiomyopathy. Adequate oral or intravenous feeding is mandatory in patients with significant weight loss, debilitating diarrhoea, absorption problems, or intestinal pseudo-obstruction. Various problems can cause diarrhoea, such as disturbed bowel motility, bacterial overgrowth, bile salt malabsorption and massive bowel wall infiltration with amyloid. Multisystem involvement results in a mix of serious problems and in such a situation it is almost impossible to find an appropriate treatment for all symptoms.[1]

EFFECT OF TREATMENT

The final step after the establishing therapy is the measurement of effect. This is especially true for patients with such an intangible disease as systemic amyloidosis.[30] The essence of the 'precursor-product' concept is that no further accumulation of amyloid deposits will occur after successful standstill of the supply of precursor proteins. Besides, the hope is that the body will be able to remove some of the amyloid deposits still present. Repeated measurements after specific time intervals can give an idea of the effect of therapy. It is important to note that two different processes should be monitored in this way.
Firstly, the underlying process with its precursor protein should be monitored: 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 10 mg/l, free kappa and lambda levels and kappa/lambda ratio should return to normal reference ranges and mutant TTR should not be detectable in the blood. Secondly, the process of amyloid accumulation should be assessed by measuring the 'amyloid load'. For this measurement quantitative abnormal clinical signs should be monitored, such as serum albumin, alkaline phosphatase, bilirubin, creatinine clearance, proteinuria, ventricular wall thickness, ejection fraction, conduction and rhythm, heart rate variability, Ewing battery results and the size 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.[30] SAP scintigraphy, if abnormal at presentation, is the method of choice to monitor amyloid load in the individual patient.[18,20,30,31] Although differences among the leading research groups are small, response criteria are currently not standardised. Comparing results of therapy will become much easier if the international amyloid community is able to create a generally accepted set of criteria for response, stable disease and progressive disease for the different types of systemic amyloidosis.

TREATMENT PERSPECTIVES

The 'precursor-product' concept focuses on the prevention of further deposition of amyloid. Clinical research is directed to developing new drugs that can interfere with amyloid deposition or can stimulate the removal of amyloid deposits. A promising new drug for patients with AA amyloidosis is sodium-1,3-propane-disulfonate (Fibrillex). This drug is a glycosaminoglycan-mimetic drug that binds to SAA. This binding may prohibit binding of SAA to glycosaminoglycans in tissue.[32] A multinational phase II/III trial started in 2001 and results are to be expected in the summer of 2005. In AL amyloidosis 4'-iodo-4'-deoxydoxorubicin (IDOX) may have effect in soft tissue involvement, although definite proof has to be awaited.[33] CPHPC is another drug that leads to depletion of SAP from the circulation.[34] If this mechanism indeed stops accumulation of amyloid, it may be very useful for all types of systemic amyloidosis. However, clinical results are not available yet. Diflunisal is worth mentioning, which might be useful as stabilising ligand in patients with ATTR amyloidosis. This drug stabilises *in vitro* the TTR tetramer in blood and prohibits its degradation into amyloidogenic dimers and monomers.[35]
A completely different approach is vaccination. Research has been focused on conformational epitopes present in all types of amyloid that might be used for vaccination.[36] If this hypothesis turns out to be valid, it can be used for patients with all types of systemic amyloidosis. What is more, in the future preventive vaccination might be considered in people at risk for the development of amyloidosis.

CONCLUSION

A systematic, stepwise evaluation of patients with systemic amyloidosis helps to get a grip on this intangible disease. Histological proof of amyloid, verification of systemic involvement, assessment of the particular type of amyloid and its precursor form the background for 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.

NOTE

Most data were presented at the Immunology symposium on Systemic Diseases in Groningen, 14 February 2003 and at the Internal Medicine Congress in Maastricht, 15 May 2003.

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Gluconeogenesis and fasting in cerebral malaria

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ABSTRACT

Background: In healthy subjects after an overnight fast, glucose production is for ~50% derived from glycogenolysis. If the fast is prolonged, glucose production decreases due to a decline in glycogenolysis, while gluconeogenesis remains stable. In cerebral malaria, glucose production is completely derived from gluconeogenesis after an overnight fast. It is not known if glucose production also decreases during fasting when its only source is gluconeogenesis.

Design: Glucose production was measured by infusion of [6,6-$^2H_2$]glucose in seven patients with cerebral malaria after prolonging a fast from 20.30 to 00.30 hours.

Results: Glucose production decreased by ~10% (27.4 ± 2.1 to 24.7 ± 1.6 μmol/kg/min, p=0.05), without changes in the plasma concentrations of glucoregulatory hormones, FFA or precursors.

Conclusions: In the patients with cerebral malaria, glucose production decreases during fasting due to a decrease in the rate of gluconeogenesis. These data suggest that the decrease in the rate of glucose production during short-term fasting is actively regulated and not simply due to shrinkage of glycogen content, as in the absence of glycogenolysis, glucose production decreases at the same rate as normally seen in healthy subjects whose glucose production is for ~50% derived from glycogen and in whom gluconeogenesis is stable.

INTRODUCTION

The adaptive response to starvation in healthy subjects involves a series of metabolic alterations. These include a decrease in the rate of glucose production by about 10 to 20% in the first 24 hours of fasting.[1,2] Glucose production has two components: gluconeogenesis and glycogenolysis. After an overnight fast both contribute more or less equally to total glucose production.[3-7] With progression of the fast the percentage contribution of gluconeogenesis to glucose production increases; the absolute rate of gluconeogenesis, however, neither increases or decreases, but remains stable for periods of up to 64 hours at the level obtained after an overnight fast.[4-9] The decrease in total glucose production with a *constant* rate of gluconeogenesis over time during fasting is not only found in healthy subjects, but also in the few studies in patients with an increased rate of gluconeogenesis.[7-9]

The change in glucose production during fasting seems merely to be a diminution in the rate of glycogenolysis, simply ascribed to a decline in glycogen content. However, in the last decades it has become clear that regulation of the rate of glucose production involves a network of regulatory systems (the classical hormones, liver glycogen content, paracrine mediators and the autonomic nervous system). Such a network suggests active regulation of glucose production during fasting and not simple dependence on glycogen content.[10] This notion is supported by studies showing that the liver is able to autoregulate between gluconeogenesis and glycogenolysis in order to fix glucose output at a set level only dependent on the duration of the fast.[11]

If this is true, it can be expected that even when glucose production is completely derived from gluconeogenesis,

glucose production during short-term fasting will diminish at the same rate as in healthy subjects, whose glucose production is for ~50% derived from glycogen. Recently we showed in cerebral malaria patients that after an overnight fast glucose production is for 100% derived from gluconeogenesis.[12] We hypothesised that if during fasting, glucose output is actively set at a level *only* dependent on the duration of the fast, glucose production will also decrease during fasting in patients whose glucose production after an overnight fast is completely dependent on gluconeogenesis.

We therefore studied glucose kinetics during fasting, using [6,6-$^2H_2$]glucose to measure glucose production in seven patients with cerebral malaria, consecutively admitted to Bao Loc General Hospital in Vietnam. As the greatest changes in glucose metabolism induced by starvation occur in the first 24 hours[1] and withholding food for prolonged periods in critically ill patients is unethical, we measured glucose kinetics during the last four hours of a ~24 hour fast.

MATERIAL AND METHODS

Subjects

Seven nonpregnant patients with cerebral malaria consecutively admitted to the intensive care unit of Bao Loc General Hospital were recruited. The inclusion criteria were based on the definition of the WHO for cerebral malaria.[13] Exclusion criteria were treatment with quinine,[14] severe anaemia (Hct <15%) and concomitant infectious disease. The study was approved by the local health authorities and by the Medical Ethics Committee of the Academic Medical Centre, Amsterdam, the Netherlands.

Study design

Patients were recruited on the day of admission after quinine use was excluded by quinine dipstick.[15] Patients were treated with artesunate intravenously according to the standard regimen of the hospital, as previously described,[12] right after laboratory confirmation of the diagnosis. After receiving informed consent signed by a first-degree relative, the patient was given a standard meal of approximately 400 to 450 ml of soup (of rice and pork meat) through a gastric tube (standard regimen for comatose patients in this hospital), followed by a fast until completion of the study. Plasma glucose concentration was measured at the bedside two hourly or hourly if a previous value was low or whenever there was suspicion of hypoglycaemia.

The time of the last meal was set at T=0. Twelve hours after administration of the meal, an intravenous cannula was introduced into a forearm vein for blood sampling. The catheter was kept patent by a slow isotonic saline drip. A blood sample for background enrichment of [6,6-$^2H_2$]glucose was drawn at T=18.30. Then a primed (3.2 mg/kg), continuous (2.4 mg/kg/h) infusion of [6,6-$^2H_2$]glucose (Cambridge Isotope Laboratories, Andover MA, USA), dissolved in sterile isotonic saline, was administered by a motor-driven, calibrated syringe pump (Perfusor® Secura FT, B.Braun, Germany) through a millipore filter (size 0.2 μm; Minisart, Sartorius, Germany). Three blood samples were collected at intervals of ten minutes at T=20.20, 20.30 and 20.40 for determination of plasma glucose concentration and [6,6-$^2H_2$]glucose enrichment. Blood samples for the measurement of plasma concentration of insulin, counterregulatory hormones, alanine, lactate, FFA, glycerol and [6,6-$^2H_2$]glucose enrichment were collected at T=20.30 and 00.30. The study ended at T=00.30.

Blood for [6,6-$^2H_2$]glucose enrichment as well as hormones was collected in pre-chilled heparinised tubes and for lactate and alanine in fluoride tubes. All samples were kept on ice and centrifuged immediately. Plasma and urine were stored at below -20°C and were transported on dry ice before assay in the Netherlands.

Assays

Plasma samples for glucose enrichments of [6,6-$^2H_2$]glucose were deproteinised with methanol.[16] The aldonitril penta-acetate derivative of glucose[17] was injected into a gas chromatograph/mass spectrometer system. Separation was achieved on a J&W (J&W Scientific, FOL, CA, USA) DB17 column (30 m x 0.25 mm, d_f 0.25 μm). Glucose concentrations were determined by gaschromatography using xylose as an internal standard. Glucose was monitored at m/z 187, 188 and 189. The enrichment of glucose was determined by dividing the peak area of m/z 189 by the total peak area and correcting for natural enrichments. The isotopic enrichments were measured on a gaschromatograph mass spectrometer (model 6890 gaschromatograph coupled to a model 5973 mass selective detector, equipped with an electron impact ionisation mode, Hewlett-Packard, Palo Alto, CA) (coefficient of variation (CV) intra-assay 2%, inter-assay CV 4%).

Plasma insulin concentration was determined by RIA (Insulin RIA 100, Pharmacia Diagnostic AB, Uppsala, Sweden), intra-assay CV was 3 to 5%, inter-assay CV 6 to 9%, detection limit 15 pmol/l; cortisol by enzyme immunoassay on an Immulite analyser (DPC, Los Angeles, CA), intra-assay CV was 2 to 4%, inter-assay CV 3 to 7%, detection limit 50 nmol/l; glucagon by RIA (Linco Research, St. Charles, MO, USA), intra-assay CV was 3 to 5%, inter-assay CV 9 to 13%, detection limit 15 ng/l; norepinephrine and epinephrine by an in-house HPLC method; norepinephrine intra-assay CV was 6 to 8%, inter-assay CV 7 to 10%, detection limit 0.05 nmol/l, epinephrine intra-assay CV was 6 to 8%, inter-assay CV 7

to 12%, detection limit 0.05 nmol/l; free fatty acids by an enzymatic method (NEFAC; Wako chemicals GmbH, Neuss, Germany), intra-assay CV was 2 to 4%, inter-assay CV 3 to 6%, detection limit 0.02 mmol/l.

Calculations and statistics

Because plasma glucose concentrations and tracer/tracee ratios for [6,6-$^2H_2$]glucose were varied (albeit only slightly) at each sampling phase of the study, calculations for non-steady-state kinetics were applied, adapted for the use of stable isotopes.[18]

Two sample comparisons were made using the Mann-Whitney test. Time points within either study were compared using a paired sample t-test. A p value ≤0.05 was considered statistically significant. SPSS statistical software programme was used for analysis.

Data are presented as means ± SEM, unless otherwise stated.

RESULTS

Table 1 shows the clinical and biochemical characteristics with cerebral malaria. All patients were admitted to the intensive care unit in a comatose state with a Glasgow coma scale less than 11. Their duration of illness was 4 ± 1 days. The current standard regimen for treatment of cerebral malaria in Vietnam (artesunate intravenously) was applied immediately after admission to the ICU. The data on glucose production in the extended fast after 20.30 hours in these patients have been published before.[12]

Glucose metabolism, precursors, free fatty acid and glucoregulatory hormones are shown in *tables 2* and *3*. Glucose production decreased over time by 10% in patients (p=0.05), without any change in the plasma concentrations of the glucoregulatory parameters.

Table 1

Clinical and biochemical characteristics of seven patients with cerebral malaria

CHARACTERISTICS CEREBRAL MALARIA PATIENTS	
Age (years)	32 ± 5
Sex (male/female)	6/1
BMI	19.3 ± 0.7
Temperature (°C)	38.2 ± 0.6
Parasitaemia (per µl)	99,849 ± 60,742
Glasgow coma score	6 ± 1
Haemoglobin (mmol/l)	7.8 ± 0.6
Serum AST (U/l)	183 ± 65
Serum ALT (U/l)	123 ± 28
Creatinine (µmol/l)	124 ± 18

Data are means ± SEM.

Table 2

Glucose concentration and glucose production in seven patients with cerebral malaria

TIME OF FASTING	20.30 H	00.30 H	CHANGE OVER TIME
Glucose concentration (mmol/l)	6.68 ± 0.31	6.57 ± 0.42	ns
Glucose production (µmol/kg/min)	27.4 ± 2.1	24.7 ± 1.6	p=0.05

Values are means ± SEM, ns= not significant.

Table 3

Precursors, glucoregulatory hormones and free fatty acid

TIME OF FASTING	20.30 H	00.30 H	P VALUE
Precursors			
Alanine (µmol/l)	286 ± 29	343 ± 94	ns
Glycerol (µmol/l)	88 ± 6	93 ± 11	ns
Lactate (mmol/l)	2.55 ± 0.82	2.32 ± 0.77	ns
Hormones			
Insulin (pmol/l)	49 ± 11	41 ± 9	ns
Glucagon (ng/l)	132 ± 33	144 ± 36	ns
Cortisol (nmol/l)	1080 ± 163	949 ± 149	ns
Norepinephrine (nmol/l)	4.8 ± 3.5	4.3 ± 3.0	ns
Epinephrine (nmol/l)	0.27 ± 0.13	0.25 ± 0.14	ns
FFA (mmol/l)	0.80 ± 0.06	0.81 ± 0.05	ns

ns= not significant.

DISCUSSION

Our data clearly show that glucose production declined significantly over time in patients with cerebral malaria when the fast was extended from 20.30 to 00.30 hours. We have previously shown that in the patients with cerebral malaria, gluconeogenesis (GNG) accounted for 100% of glucose production at T=20.30.[12] Therefore the decline in glucose production during the prolongation of the fast was due to a decline in the rate of gluconeogenesis. This finding is not remarkable for the decrease in glucose production, as numerous studies have shown that in healthy subjects glucose production decreases by ~10% between 16 and 22 hours of fasting, due to a decrease in the rate of glycogenolysis.[1,2] The finding is remarkable for the decrease in the rate of gluconeogenesis.

One could postulate that in the case of 100% gluconeogenesis, glucose molecules formed during gluconeogenesis could lead to an underestimation of the glucose production because of the exchange of H on the C6 position with

deuterium from the water. However, during gluconeogenesis not only the H-atoms on the 6th position but also the ones on the C5 and the C2 will be labelled, thus leading to a glucose molecule with m+3 or m+4. In our [6,6-$^2H_2$] glucose analysis for measurement of glucose production only the fragment of m+2 with all six C-atoms is measured making this confounding variable unlikely.

The decrease in the rate of gluconeogenesis over time in our patients with cerebral malaria is remarkable. The gold standard for measurement of gluconeogenesis in humans *in vivo* is either deuterated water or NMR.[19] All existing data indicate that gluconeogenesis, measured with these techniques, is *constant* during fasting for periods up to 64 hours even in diabetic patients with an increased rate of gluconeogenesis.[3-7,9] Gluconeogenesis is dependent on precursor supply and the glucoregulatory hormones.[9,12,20] The plasma concentrations of the precursors and the glucoregulatory hormones did not differ between T=20.30 and T=00.30 and therefore do not explain the decrease in glucose production over time in our patients. A potential explanation for this decrease in production in our patients could be *active* regulation of the rate of total glucose production (independent of its source, either glycogen or gluconeogenesis) in relation to fasting time instead of a decrease in glucose production simply caused by shrinkage of glycogen content. This hypothesis of active regulation is supported by recent data from our group.[21] In that study we measured glucose production twice in healthy humans, fasting from 16 to 22 hours, once during infusion with a low dose of insulin in an amount that increased plasma insulin slightly above the basal level and another time without infusion of insulin (control study). In the control study, glucose production declined by ~18%. Within the first hour of insulin infusion a significant decline in glucose production was found compared with the control study. Subsequently a rebound increase in glucose production was found. From the third hour of insulin infusion onwards glucose production was no longer different between both study conditions. These changes in glucose production during low-dose insulin occurred without changes in the concentrations of the counter-regulatory hormones. This observation and the observation that the liver is able to autoregulate between gluconeogenesis and glycogenolysis suggest that during short-term fasting glucose production is set at a certain level, a level that changes (diminishes) over time with progression of the fast.[11] Data in healthy subjects suggest that this regulation is primarily directed at glycogenolysis.[6] Our data in patients with cerebral malaria suggest that when direction at this primary target is impossible, this regulatory process will be targeted at the rate of gluconeogenesis.

The nature of this regulatory process can not be inferred from this study. Glucoregulatory hormones and free fatty acids do not seem to play a role. A role for the autonomic nervous system is also less likely, although the influence of the sympathetic nervous system during a prolongation of the fast to 24 hours has not been studied.[22,23] Therefore, the available data point to a paracrine network in the liver itself that exerts a potent glucoregulatory role during fasting.[10]

We conclude that in cerebral malaria, the decline in glucose production during short-term fasting is due to a decrease in the rate of gluconeogenesis. We hypothesise that in humans the decrease in rate of glucose production during short-term fasting is actively regulated, only dependent on the duration of the fast, and not simply due to shrinkage of glycogen content, as in the absence of glycogenolysis, glucose production decreases at the same rate as normally seen in healthy subjects, whose glucose production is for ~50% derived from glycogen and in whom gluconeogenesis is stable.

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CASE REPORT

Meningococcal pericarditis and tamponade

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ABSTRACT

We report the case of a 37-year-old female with a complex manifestation of serogroup C meningococcal disease. The patient presented with symptoms and signs of pneumonia, sepsis and diffuse intravascular coagulation. Moreover, she suffered from a culture-proven pyogenic pericarditis that deteriorated into cardiac tamponade. Immediate pericardiocentesis was successful and eventually the patient recovered.

INTRODUCTION

Meningococcal disease is caused by *Neisseria meningitidis*, a gram-negative diplococcus. Humans are the only natural hosts in which meningococci are pathogenic.[1] The most common clinical manifestations of meningococcal disease are meningitis, sepsis and concurrent pneumonia. Much less frequent manifestations are conjunctivitis, arthritis, urethritis, pericarditis, otitis media, sinusitis and epiglottitis. A rare manifestation is chronic meningococcaemia.[2-4] We report a case of a 37-year-old female with a complex manifestation of meningococcal disease, including pyogenic pericarditis. In the months before her illness the incidence of meningococcal disease, caused by *N. meningitidis* serogroup C, had increased markedly in the Netherlands.[5]

CASE REPORT

A 37-year-old female with a history of borderline personality disorder was suffering from dyspnoea, dry cough, fever, episodes of palpitations and nausea. Her general practitioner suspected a pneumonia and prescribed amoxicillin 500 mg orally three times a day. The patient had no other medication. Her condition, however, did not improve and on the 4th day of her illness she was referred to our hospital.
On arrival the patient's condition was critical. She was tachypnoeic and cyanotic. Her blood pressure was 90/50 mmHg, pulse 130 beats/min and temperature 38.2°C. External jugular veins were distended and the central venous pressure was 23 cm of water. At cardiac auscultation S1 and S2 were soft, neither murmurs nor friction rub were heard. Dull percussion and bronchial breath sounds were found at the base of both lungs. The liver was slightly enlarged and tender. The extremities were cool and pale with mottling. Petechiae were absent at this stage. Neurological examination was normal.
The electrocardiogram demonstrated sinus tachycardia, low QRS voltage and slight negative T waves in leads V_1 to V_3. There was no electrical alternans. Chest X-ray showed an enlarged cardiac silhouette, consolidations in the inferior lobes of both lungs and pleural effusion. Laboratory investigation revealed a C-reactive protein of 278 mg/l, a leucocyte count of 26 x 10^9/l with left shift, a haemoglobin of 6.9 mmol/l, and a thrombocyte count of 106 x 10^9/l. INR was 2.50, fibrinogen 6.7 g/l, and D-dimer >2000 μg/l. The plasma creatinine was 761 μmol/l.
The differential diagnosis consisted of pneumonia and sepsis of unknown origin, complicated by renal failure and diffuse intravascular coagulation (DIC). Antibiotic therapy was started, consisting of amoxicillin, clavulanic acid and tobramycin intravenously.
Pericardial effusion was suspected because of the enlarged

cardiac silhouette, low-voltage ECG and elevated central venous pressure. Echocardiography confirmed a large volume of pericardial effusion and showed compression of both the right ventricle and the right atrium. Pericardiocentesis was performed which yielded 700 ml of viscous, straw-coloured fluid. Gram stain of the pericardial fluid showed small gram-negative diplococci and the leucocyte count was 128.8 x 10^9/l. The presumptive pathogen was *N. meningitidis* and antibiotic therapy was changed to penicillin G, 4 million units intravenously every six hours. After the pericardiocentesis the patient improved at once, the mottled aspect of her skin disappeared and diuresis recovered. A drainage catheter was left in the pericardium for two days.

Culture of the pericardial fluid and immunological reactivity identified *N. meningitidis*, serogroup C, serotype 2a, with good antibiotic susceptibility for both penicillin G (MIC 0.032 mg/l) and amoxicillin. Blood cultures taken before the initiation of the parenteral antibiotic therapy remained negative. A lumbar puncture yielded normal liquor and no pathogens on gram stain and culture. Cultures of expectoration did not reveal a specific pathogen. Serological examination showed a *Mycoplasma pneumoniae* IgM antibody titre greater than 1:20480. Cultures from urethral and vaginal smears excluded *Neisseria gonorrhoeae*.

The day after pericardiocentesis the patient's condition worsened because of ongoing sepsis and aggravation of the DIC (INR 3.11, thrombocytes 60 x 10^9/l), and petechiae and ecchymoses developed on the lower extremities. Low-dose heparin, extensive fluid and inotropic therapy were given. Unfortunately necrosis of both lower extremities developed, which resulted in amputation of both her right lower leg and three toes of her left foot. Eventually she recovered well.

DISCUSSION

Our patient presented with a clinical picture of sepsis due to meningococcal disease. The site of infection appeared to be a pericarditis. Invasive meningococcal disease can present in different ways. The most benign presentation is the transient meningococcaemia, the worst presentation is called the fulminant meningococcal sepsis.

Primary meningococcal pericarditis (PMP) is a rare presentation of meningococcal disease. PMP should not be confused with the reactive, immune complex-mediated pericarditis, which occurs in 10 to 20% of patients during the convalescent phase of invasive meningococcal disease.[6] PMP is mainly caused by *Neisseria meningitidis* serogroup C. This bacterium also caused the PMP in our patient.[7]

Interesting in our case is the way the patient presented. DIC was present on the day of admission and resulted in limb ischaemia for which amputation was necessary. This presentation of PMP is quite exceptional. PMP usually presents as a febrile disease without severe sepsis or DIC and usually runs a relatively benign course with an excellent prognosis, provided that appropriate antibiotics are given and, if necessary, pericardiocentesis or surgical release is performed. The meningococcus reaches the pericardium via the haematogenous route and the pericarditis is usually the only infectious site. Blood cultures are negative in more than half of the patients. Our patient also had negative blood cultures but she was treated with amoxicillin orally for four days before hospital admission.[7-10]

Serological examination indicated that the patient had recently suffered from an *M. pneumoniae* infection. This infection probably facilitated meningococcal invasiveness by diminishing the barrier function of patient's respiratory mucosa.[11,12] Caution should be taken when interpreting the serological test for *M. pneumoniae* because cross-reactive antibodies appear to play a role in positive results found in patients with other bacterial infections.[13]

CONCLUSION

This case report shows that PMP can present as a severe ongoing sepsis complicated by DIC that can even lead to severe ischaemia of the extremities.

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CASE REPORT

Deep vein thrombosis associated with distension of the urinary bladder due to benign prostatic hypertrophy - a case report

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ABSTRACT

A 76-year-old man was admitted with a first episode of deep vein thrombosis (DVT) of his left leg. It was associated with a distended urinary bladder, due to benign prostatic hypertrophy. Screening for malignancy was negative. Laboratory testing revealed protein S deficiency. Although a distended bladder may induce venous stasis, it is not a proven risk factor for DVT. Clinical expression possibly depends on the concomitance of other risk factors, such as inherited or acquired thrombophilic defects. However, it is also possible that the association of a distended bladder with DVT of a lower limb has not been recognised yet. As a distended bladder is rather common in elderly men, a proper study is warranted to estimate the prevalence of associated DVT.

INTRODUCTION

Deep vein thrombosis (DVT) can occur due to external compression of the pelvic veins by a malignant or benign tumour. Particularly in case of a benign tumour, venous stasis is the supposed mechanism to explain DVT. However, benign tumours have rarely been reported in association with DVT.

A distended urinary bladder is diagnosed in 0.8% of elderly men annually[1] and may induce venous stasis.[2,3] Remarkably, only three case reports have been published that described a possible relation with DVT.[4-6] Here we describe another patient in whom a causal relationship between a distended bladder and DVT seemed to be plausible. However, the DVT in this patient could at least partly or even completely been explained by the concomitant presence of protein S deficiency.

CASE REPORT

A 76-year-old retired gynaecologist was admitted to our hospital with a one-day history of moderate swelling and a heavily prickling sensation of the left leg. His tentative diagnosis was DVT. It was confirmed by compression ultrasonography, which showed a thrombus in the left femoral and popliteal veins.

On the day of admission he developed acute urinary retention. He had accepted the mild micturation problems, which had already been present for a while, as normal for his age. Previous exposure to risk factors for venous thromboembolism had occurred when surgery for otitis media and sinusitis was performed at an age of 19 and 66 years, respectively. He was physically active and not obese. He had no history of either venous thromboembolism or any other disease. His mother experienced idiopathic DVT when she was 35 years old. She and one of her sisters died of colon carcinoma at an older age. The only abnormal findings on physical examination of the patient, apart from a swollen left leg, were a very enlarged bladder and a large prostate without palpatory signs of malignancy. The results of routine laboratory tests were within normal ranges, including haemoglobin level, white blood cell and platelet counts, and serum levels of electrolytes, urea nitrogen and creatinine. The D-dimer plasma level was 4804 μg/l (normal range 70 to 500), the serum level of prostatic specific antigen 2.4 μg/l (normal <6.5 μg/l). Abdominal echography revealed an overfilled urinary bladder without distension of the pyeloureteral systems. A Foley catheter was introduced suprapubically into the bladder and about 2.5 litres of clear urine was evacuated. Echography, chest X-rays and colon X-rays provided no evidence of a malignant tumour.

A transurethral resection of the prostate was performed four months later. Histology of the removed prostatic tissue showed benign hypertrophy.

Treatment of the DVT consisted of subcutaneous tinzaparin for seven days and acenocoumarol for three months. Because the patient was not convinced that venous stasis by the distended urinary bladder was a sufficient explanation for DVT, he requested laboratory testing of thrombophilic defects. Protein S deficiency was demonstrated at repeated measurements. Free protein S plasma levels amounted to 37 and 46%, respectively (normal range 76 to 120%) in samples that were collected four months after the acute DVT, when he was no longer receiving acenocoumarol. Plasma levels of antithrombin (87%), protein C (84%) and plasminogen (98%) were within normal ranges. Lupus anticoagulant and anticardiolipin antibodies were not detectable. DNA analysis did not reveal factor V Leiden or the prothrombin G20210A mutation.

DISCUSSION

A distended bladder can compress the iliac veins. Hopkins *et al.* demonstrated in six volunteers with normal bladder capacity (400-600 ml) that the pressure in the femoral veins rose significantly when the bladder contents approximated 300 ml, although variations were observed.[2] The pressure elevation was on average much higher in five patients with urinary retention due to benign prostatic hypertrophy and a bladder capacity that ranged from 1000 to 2200 ml. Three of these patients also had oedema of the lower limbs. They had the highest pressure elevation, which was even greater than 50 cm H_2O. Similar results were reported by Nilsson *et al.* during transurethral resection of the prostate when the urinary bladder was intermittently filled with irrigating fluid.[3] In both studies, measurements were performed in supine position. Compression of the iliac veins due to bladder enlargement is expected to occur rather frequently in elderly men with benign prostatic hypertrophy. It may lead to oedema of one or both legs,[7,8] comparable with oedema of the legs in women during the last trimester of pregnancy as a result of compression by the uterus.

Venous stasis is a generally accepted predisposing factor for DVT, as Virchow already postulated in 1865.[9] It is therefore remarkable that only three cases have been reported since 1993, in which DVT of a lower limb was associated with a distended urinary bladder,[4-6] as the latter condition is rather common in elderly men. Unfortunately, no information was given about other thrombotic risk factors in the three patients mentioned above. Based on the few cases reported in the literature, a distended urinary bladder does not seem to be, or is only a mild, risk factor, clinical expression of which depends on the concomitance of other risk factors, such as the thrombophilic defect in our patient. Such a conditional causal relationship is in accordance with the current view that venous thromboembolism is often a multicausal disease.[10] However, even then, a higher prevalence of DVT in elderly men would be expected, considering the increasing number of more or less prevalent inherited or acquired thrombophilic defects that have been identified as risk factors.[10] One can therefore speculate about a protective mechanism against the development of DVT, such as an increased release of urokinase-type plasminogen activator from the distended bladder wall.[11]
In conclusion, a distended urinary bladder may compress the iliac veins but is surprisingly very seldom associated with DVT of a lower limb. Apparently, other thrombotic risk factors are required to express venous thrombosis in this condition, such as the presence of protein S deficiency in our patient.

In our opinion, a prospective study is warranted to obtain an accurate risk estimate of venous thrombosis in consecutive patients with an urinary bladder distension to assess its clinical implications.

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ANSWER TO PHOTO QUIZ (ON PAGE 119)
TWO PATIENTS WITH RECURRENT FEVER AND WINE RED DISCOLOURATION OF THE EYELIDS

DIAGNOSIS

At the time of presentation the recurrent febrile attacks had been present for several decades in both patients. Such a long duration makes an infectious or neoplastic disorder unlikely.[1] The attacks tend to recur after an asymptomatic period and have a predictable course, with a similar set of symptoms and time course during each attack. This suggests a form of periodic fever. Onset of the first attack in early childhood with lifelong persistence and the fact that more family members are affected suggests a congenital or hereditary form of periodic fever. Because women as well as men are affected an autosomal inheritance pattern is likely. Two of the four main subtypes of hereditary periodic fever syndromes show an autosomal recessive inheritance pattern: familial Mediterranean fever (FMF) and the hyper-IgD syndrome (HIDS). TNF-receptor associated periodic syndrome (TRAPS) and the cryopyrin-associated periodic syndromes (such as the Muckle-Wells syndrome) show an autosomal dominant inheritance pattern.[1,2] In this family, an autosomal dominant inheritance is likely. Because none of the family members have experienced urticaria (a distinctive feature of the cryopyrin-associated syndromes), the most likely diagnosis would be TRAPS. TRAPS is caused by mutations in the gene encoding for the TNF type 1 receptor (TNFRSF1A). These mutations are thought to cause increased signalling of TNF-β, thus inducing cytokine secretion, activation of leucocytes, fever and cachexia. The attacks in TRAPS are characterised by spiking fever, skin lesions, myalgia, arthralgia, abdominal distress and ocular symptoms including peri-orbital oedema as shown by the picture (*figure 1*).[1,2] In this family we found a missense mutation (C29F) of the gene encoding for the TNF type 1 receptor in all affected family members, confirming the diagnosis of TRAPS. After the diagnosis was confirmed both patients were treated with etanercept, a recombinant form of the TNF type 2 receptor, which blocks TNF signalling. With this treatment both intensity and duration of the symptoms were reduced.

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