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EDITORIAL

Are prognostic factors in rheumatoid arthritis of any use in daily clinical practice?

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Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of unknown origin with a highly variable presentation. Its main manifestation is synovitis of the peripheral joints. The disease usually starts in the small joints of the hands and then gradually develops in the feet; all the other, larger joints may become involved as well. This does not only cause the patient a great deal of discomfort, like pain and stiffness, but also has a huge impact on mobility and psychosocial wellbeing. The course of RA is very heterogeneous. Some patients undergo a mild course that may resolve within months or years, often without any structural damage while others have severe, erosive disease with extra-articular manifestations and decreased life expectancy. Pharmacotherapy is still the cornerstone in the management of RA, a distinction being made between first- and second-line treatment. First-line treatment, i.e. non-steroidal anti-inflammatory drugs (NSAIDs), is given as soon as the symptoms of pain and stiffness appear, while second-line agents are usually given only after the diagnosis RA has been confirmed.

In the past decade many new treatments have become available for the management of RA, including leflunomide and the biological agents.[1,2] This has changed the treatment strategy dramatically: patients are now being treated earlier and more aggressively. Some of these treatments are very toxic and/or expensive. In order to improve the risk/benefit ratio of the pharmacotherapy, many attempts have been made to find factors which could predict the course of the disease.[3] If that were possible, only those patients in whom the disease is expected to run a severe course would be offered the most effective treatment, which is often also more toxic and/or expensive.

Many factors have been described that predict joint destruction and functional disability in patients with RA. Probably the most useful are those factors that are independent of disease activity, such as the presence of rheumatoid factor and the so-called shared epitope of HLA-DRB1. The early presence of bony erosions is another important prognostic marker. In addition, clinical indicators such as many affected joints, the presence of extra-articular features and a considerable degree of physical disability at onset are associated with poor prognosis, as are sociodemographic markers such as older age at onset and a lower level of formal education.

In this issue Van Venrooij and colleagues discuss the properties of a new specific autoantibody: the anticyclic citrullinated antibody (anti-CCP).[4] In a long-term follow-up study of patients with recent onset RA it was shown that patients positive for this antibody had significantly more severe joint destruction than the anti-CCP negative patients.[5] However the additional predictive value over the IgM rheumatoid factor test was only modest.

In conclusion: although several factors have been shown to be able to predict a more severe disease course, their positive predictive value is still not strong enough to be useful in daily clinical practice.

Another characteristic of the anti-CCP test is a higher specificity compared with the IgM rheumatoid factor test. As the IgM rheumatoid factor, anti-CCP antibodies are frequently present many years before the diagnosis of RA can be made. Due to these two features the anti-CCP test may have an important role in the early diagnosis of RA. As many studies have shown that therapeutic interventions early in the course of the disease lead to earlier disease control and therefore less joint damage, it is

important to make the diagnosis of RA in a patient with joint symptoms as soon as possible.[6] The classification criteria developed by the American College of Rheumatology have been used to do this, although they were not designed for this purpose.[7] These criteria were originally developed in an established patient population to classify RA in order to be able to compare different patient populations. So, these criteria are not the optimal instrument to distinguish early RA from undifferentiated polyarthritis. Van Venrooij and colleagues demonstrate that it is possible to discriminate erosive versus non-erosive arthritis or self-limiting from persistent arthritis using the anti-CCP test in a prediction model including six other variables. The discriminative ability of the same model without the anti-CCP test was significantly lower. The differences, however, were remarkably small.

Although these findings are of great importance for basic and clinical research in RA, the question remains what the consequences are for our daily clinical practice. Should we test all our patients with an early undifferentiated arthritis and rheumatoid arthritis and treat them aggressively in case of a positive anti-CCP test? Is there still a need for clinical joint examination, laboratory tests as the acute-phase response and imaging of the joints by regular X-rays of hands and feet? Yes, certainly there is!

One baseline assessment of disease activity is not sufficient to predict the future course of the disease, although several studies have shown that time-integrated disease process variables do reflect the outcome of the disease. We all know that persistent high disease activity causes many immediate problems to the patient, but it has also been shown that this is more likely to eventually lead to irreversible joint damage,[8] a higher probability of developing secondary lymphomas[9] and even a reduction in life expectancy.[10] Disease-controlling antirheumatic therapies do influence the disease activity,[11] therefore to guide treatment decisions it is important to follow the fluctuating course of the disease activity as accurately as possible.[12] In fact, this is no different from monitoring the glucose level in patients with diabetes mellitus and the blood pressure in patients with hypertension.

Due to the heterogeneity of the disease expression, it is not possible to assess disease activity in all patients with rheumatoid arthritis with one single variable. Disease activity should be represented by a set of variables, which can be reported and analysed either separately or as part of an index of disease activity like the DAS28.[13] Serial measurements of the DAS28 have shown to be strong predictors of physical disability and radiological progression. Beside variables assessing disease activity, which should be measured frequently, joint damage should be monitored periodically with X-rays, and possibly also functional capacity with a patient questionnaire, to follow the disease process in the long term. The anti-CCP test has a role in the early detection of the disease. The decision to start or change antirheumatic therapies, however, is still based on the complete clinical picture of the patient. The role of prognostic factors in this respect is only modest.

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REVIEW

Anticitrullinated protein/peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis

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INTRODUCTION

Rheumatoid arthritis (RA) is considered a systemic autoimmune disease with the main characteristic of persistent joint inflammation that results in joint damage and loss of function. Numerous studies have shown that substantial irreversible damage occurs within the first two years, as evidenced by the maximal rate of erosive joint disease that appears early on.[1,2] There is growing evidence that therapeutic intervention early in the disease course of RA leads to earlier disease control and less joint damage.[3-7] Moreover, in the last years there has been a rapid development of powerful therapeutic agents for RA.[8] Rheumatoid arthritis should be considered a medical emergency that requires prompt diagnosis and appropriate treatment.[7,9] On the other hand, many early arthritis patients not diagnosed as RA have self-limiting disease.[10] Since treatment of early arthritis with disease-modifying antirheumatic drugs (DMARDs) is only justified when the cost-benefit ratio is favourable, it is mandatory to be able to differentiate between RA and other forms of arthritis early after symptom onset.[7,11] Therefore, diagnostic criteria for RA that are maximally accurate and at the same time usable in clinical practice are needed. In this context it would be extremely helpful to have a simple serological marker that is highly specific for RA, present early in disease and prognostic as to whether the disease will be erosive or not. The search for such an ideal serological marker of RA that could be included in the diagnostic criteria has been going on for decades, but only recently appears to have yielded results, as will be discussed below.
In this review we will focus on diagnostic criteria for early RA and the possible role herein of specific autoantibody activities.

EARLY ARTHRITIS

The term early arthritis is often used in literature but is not well defined. From a practical clinical point of view early arthritis could be defined as arthritis newly presented to a clinician that poses a diagnostic, prognostic and therapeutic challenge. Early arthritis patients constitute a very heterogeneous group of patients, both as to their clinical presentation and their outcome. Depending on the way these patients are selected, about one third of the patients have a disease that may ultimately be classified as RA, one third will have another classifiable inflammatory disorder and one third of the patients remain unclassified.[10]

ACR 1987 CLASSIFICATION CRITERIA FOR RA

As diagnostic criteria

The 1987 American College of Rheumatology (ACR; formerly, American Rheumatism Association) classification criteria for RA are shown in *table 1*. In clinical practice these criteria are often used as a diagnostic tool for RA. However, these criteria were developed in a population of selected RA and non-RA patients as a means of classifying RA, not as a way to diagnose RA.[12] This probably explains the poor diagnostic performance of the ACR criteria in early arthritis.

As a gold standard

A problem to be dealt with in the diagnostic research of RA is the lack of an independent gold standard for the disease. In most studies the disease classification according to the ACR criteria has been used as the gold standard. A draw-

Table 1
The American College of Rheumatology 1987-revised criteria for the classification of rheumatoid arthritis (traditional format)

1	Morning stiffness of at least one hour before maximal improvement
2	Arthritis of three or more joint areas
3	Arthritis of hand joints
4	Symmetric arthritis
5	Rheumatoid nodules
6	Rheumatoid factor (RF) positivity
7	Radiographic changes on hand and wrist radiographs (erosions or decalcification)

For classification purposes, a patient will be said to have rheumatoid arthritis if he/she has satisfied at least four of these seven criteria. Criteria one to four must have been present for at least six weeks.

back of this gold standard is that it is dependent on the diagnostic tests that are evaluated. This leads to circularity and overestimation of the diagnostic properties of these tests. Another drawback is that one third of the patients with persistent arthritis do not fulfil any of the international classification criteria. For the clinician it is unclear how these unclassifiable forms of persistent arthritis should be treated.
Defining the gold standard of RA in terms of arthritis outcome prevents the occurrence of circularity.[13] Moreover, predicting the outcome of arthritis is more relevant for therapeutic decision-making than predicting whether arthritis will ever satisfy a set of classification criteria. Clinicians now have several powerful drugs at their disposal that will improve outcome when applied at an early stage of the disease but also have high toxicity profiles or are expensive. Treatment with these drugs is only justified when the cost-benefit ratios for individual patients are favourable. Both for the patient and the clinician confronted with early arthritis, the knowledge of arthritis outcome is therefore indispensable for their choice of management strategies.

AUTOANTIBODIES IN RA

In systemic autoimmune diseases many autoantibodies directed to ubiquitously expressed antigens are made, and they often show restriction with respect to the autoimmune disease in which they occur. Two examples are:
1) Sm (the 'Smith' autoantigen) is a complex of eight proteins associated with a number of small RNAs present in the nucleus of every eukaryotic cell, including yeast. Nevertheless, it is targeted by autoantibodies almost exclusively produced by SLE patients.
2) The Jo-1 autoantigen is identical to His-tRNA synthetase which is an essential cofactor in the synthesis of proteins. It is therefore present in every eukaryotic cell. Notwithstanding that, autoantibodies to Jo-1 are very typical for myositis.
RA is diagnosed primarily on clinical manifestations and serological support has, up to now, been restricted to the determination of (IgM) rheumatoid factor (RF). However, this antibody, directed to the Fc part of IgG, is not specific for RA because it also occurs in many inflammatory diseases as well as in (elderly) healthy individuals. Most other published autoantibody systems in RA were also shown to occur in more than one rheumatic disease, and thus are not specific for RA.[14] Recently, however, a novel and very specific autoantibody system for RA has been described. It was found that patients with RA develop antibodies to, as yet undefined, proteins containing modified (citrullinated) arginine residues. It has been shown convincingly that the citrulline residues are essential parts of the antigenic determinants recognised by the RA autoantibodies.[15,16] The citrulline moiety in the antigen is so important that essentially every citrullinated peptide or protein will be recognised by autoantibodies in RA sera, albeit with different sensitivities and specificities.

Citrullination: what, where and when

Citrullination, or deimination, is an enzyme-catalysed process in which the positively charged NH_2-group of the amino acid arginine (Arg) is hydrolysed to a neutral oxygen group (*figure 1*). It is this oxygen group of peptidyl citrulline that is specifically recognised by autoantibodies in RA.[14-17] Database searches reveal the existence of four human peptidylarginine deiminases (PAD enzymes), but not much is known about their substrate specificity, their cellular localisation, and how and when these enzymes become activated. What we do know is that these enzymes have a tissue-specific distribution and that, in mice, they are stimulated by female sex hormones.[18]

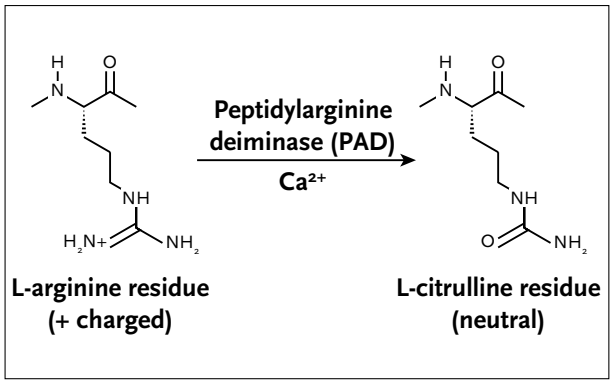

Figure 1
Deimination of peptidyl arginine to peptidyl citrulline by peptidylarginine deiminases

There are only a few citrullinated proteins known to occur in healthy mammalian cells. It is unlikely that one of these (for example myelin basic protein, filaggrin or trychohyalin) would be the citrullinated RA-specific autoantigen, since none of these proteins can be detected in, for example, synovial tissue. However, it appears that citrullinated proteins can be generated during the final stages of the lifecycle of some cells. For example, filaggrin becomes citrullinated during late differentiation of epidermal cells.[19] Vimentin and histones are citrullinated during programmed cell death (apoptosis) of macrophages and HL-60 cells, respectively, and fibrin is citrullinated in inflamed joint tissue.[20-22] Especially the presence of citrullinated fibrin in the inflamed joint is interesting, since it has been shown that the inflamed synovial tissue is the local site where the anticitrullinated protein antibodies are produced. First of all, Masson-Bessière and co-workers found that the titres of IgG antibodies directed to citrullinated protein were several times higher in the pannus tissue than in synovial fluid or serum.[23] Secondly, the titres of such antibodies in synovial fluid are also significantly higher than in paired serum samples (E. Vossenaar, unpublished data). Thirdly, B cells from the synovial fluid of RA patients with anticitrullinated protein antibodies spontaneously produce these antibodies, while peripheral blood B cells or B cells from seronegative RA patients do not.[24] These results not only suggest an antigen-driven maturation of anticitrullinated protein-specific B cells at the site of inflammation in RA, but also indicate that the production of these antibodies is a local process occurring in the inflamed synovium.

Anti-CCP antibodies

In principle, every citrullinated protein or peptide can be used in serological tests to detect anticitrullinated protein antibodies. So far, only citrullinated filaggrin has been used to detect the so-called antifilaggrin antibodies (AFA).[25] In our first attempts to find suitable substrates for RA autoantibodies we developed a number of linear peptides containing one citrulline residue. These citrullinated peptides were specifically recognised by the RA autoantibodies and, more important, their arginine-containing counterparts were not. However, most peptides reacted with only 30 to 45% of the RA sera, although more than 75% of RA sera reacted with at least one of the nine peptides tested.[15] We tested several parameters to increase the sensitivity of the test, and found that the most successful optimisation was to make the peptides cyclic. Our cyclic citrullinated peptides (CCP) have a three-dimensional design that is optimally structured for recognition of the antigenic group by the heterogeneous population of RA autoantibodies. By using a single CCP as antigen in an ELISA test, we could increase the sensitivity of the assay to about 68%, with a specificity of more than 97%.[26]

Recent selections from dedicated peptide libraries yielded novel peptides with improved recognition properties. Using such peptides, the sensitivity of the test can be increased to at least 80%, with a specificity of >98% (see CCP2 test, *table 2*).[27]

Table 2

Sensitivity and specificity of the anti-CCP2 test compared with the IgM-RF test

	CCP2			IGM-RF		
	N	POS	%	N	POS	%
RA (chronic)	390	320	82	390	312	80
Healthy individuals	95	1	1	95	1	1
Various connective tissue diseases[a]	299	9	3	264	40	15
Osteoarthritis	29	0	0	27	1	4
Reactive arthritis	40	1	3	40	4	10
Various inflammatory disease[b]	113	1	1	113	2	2
Various viral infections[c]	117	0	0	106	13	12
Various bacterial infections[d]	118	1	1	118	11	9
Various parasitic infections[e]	93	2	2	93	20	22
	809	14	2	761	91	12

[a] = including systemic lupus erythematosus, scleroderma, primary Sjögren's syndrome, vasculitis, [b] = including Crohn's disease, colitis ulcerosa, [c] = including Epstein-Barr, Parvovirus B19, [d] = including Treponema pallidum (syphilis), Chlamydia trachomatis, Legionella, Borrelia, Yersinia, Salmonella, Streptococcus pyogenes, Mycobacterium tuberculosis, [e] = including , Toxoplasma, Plasmodium falciparum (malaria), Leishmania, Schistosoma, Trypanosoma cruzi.

RECENT STUDIES USING THE ANTI-CCP SYSTEM

A simple, specific and quantitative ELISA test using a single cyclic citrullinated peptide (cfc1-cyc2) as immunosorbent has been developed and released on the market as the anti-CCP1 test (Immunoscan RA).[28] The studies performed with this test allow the following conclusions to be made.

Anti-CCP antibodies are extremely specific for RA

Various groups of researchers testing different cohorts of RA patients reached a specificity varying between 96 and 99%.[26,27-33] The anti-CCP test is thus clearly more specific that the RF test (see also *table 2*). The extreme specificity of the anti-CCP antibody system will be a great help in the early diagnosis and earlier treatment of this disease.

Sensitivity of anti-CCP and RF test is comparable

The first generation anti-CCP test had a sensitivity of 60 to 68%, somewhat lower than the RF test (70 to 75%). The

second generation CCP2 test[27,28] uses other citrullinated peptides that raises the sensitivity to 75 to 80% (*table 2*). It has appeared from various, as yet unpublished, studies of different cohorts of patients that the sensitivity of the CCP2 test is very comparable with that of the IgM-RF test.[27] In both the CCP1 and the CCP2 test about 35 to 40% of the RF-negative patients scored positively for anti-CCP.

Anti-CCP antibodies have prognostic value

This is because they are predominantly present in patients with erosive disease. Although only a few studies on the prognostic abilities of these antibodies have been performed so far, the studies by Van Jaarsveld *et al.*,[34] Kroot *et al.*,[31] Visser *et al.*,[35] and Vencovský *et al.*[32] support the idea that RA patients positive for anti-CCP develop significantly more severe radiological damage than anti-CCP-negative patients. Additional studies are necessary to further underline the prognostic ability of this test.

Anti-CCP antibodies are present very early in the disease

In studies of patients from early arthritis clinics[26,31,32,35] as well as cohorts of patients with early synovitis,[30] anti-CCP antibodies were present in 40 to 70% of the cases. In several yet unpublished studies the antibodies were detected up to ten years before the first RA symptoms were noted. These results indicate that citrullination of synovial antigens and the production of antibodies to these citrullinated antigens is initiated very early in disease.

A PREDICTION MODEL FOR PERSISTENT (EROSIVE) ARTHRITIS

In a recent study a clinical model was described for the prediction of three forms of arthritis outcome: self-limiting, persistent non-erosive and persistent erosive arthritis.[35] The prediction model was developed in a cohort of 524 early arthritis (EA) patients derived from the Leiden Early Arthritis Clinic. Outcome was determined at two years. A schematic representation of the study design is shown in *figure 2*. The developed prediction model is shown in *table 3* and consists of seven variables: symptom duration, morning

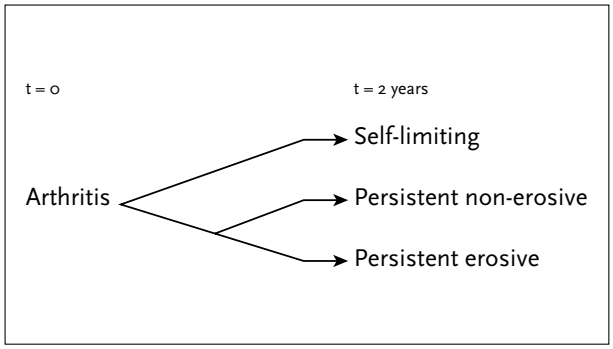

Figure 2
Schematic representation of the study design

The development of diagnostic criteria to discriminate at the first visit between three forms of arthritis outcome recorded at two-year follow-up: self-limiting arthritis, persistent non-erosive arthritis and persistent erosive arthritis.[35]

Table 3
The seven variables of a prediction model for persistent (erosive) arthritis

	PERSISTENT ⟷ SELF-LIMITING		EROSIVE ⟷ NON-EROSIVE GIVEN PERSISTENCE	
	ODDS RATIO	SCORE	ODDS RATIO	SCORE
Symptom duration				
≤6 weeks <6 months	2.49	2	0.96	0
≥6 months	5.49	3	1.44	0
Morning stiffness ≥1 hour	1.96	1	1.96	1
Arthritis ≥3 joint groups	1.73	1	1.73	1
Bilateral compression pain MTPs	1.65	1	3.78	2
IgM RF ≥5 IU	2.99	2	2.99	2
Anti-CCP1 ≥92 IU	4.58	3	4.58	3
Erosions X-rays hands or feet	2.75	2	Infinite	Infinite

Intercept persistent versus self-limiting = -2.31, intercept erosive versus non-erosive given persistence = -2.42, MTPs = metatarsophalangeal joints, RF = rheumatoid factor, CCP = cyclic citrullinated peptide. For each variable two odds ratios and two simplified scores are shown, one for its association with persistent arthritis and one for its association with erosions given arthritis is persistent.[35]

stiffness of at least one hour, arthritis of three or more joints, bilateral compression pain of metatarsophalangeal joints (MTPs), RF positivity, anti-CCP1 positivity and the presence of erosions on radiographs of hands or feet. The odds ratios of the variables are shown in *table 3* both for self-limiting versus persistent arthritis and for erosive versus non-erosive arthritis. Application of the model in an individual patient results in three clinically relevant predictive values: one for self-limiting arthritis, one for persistent non-erosive arthritis and one for persistent erosive arthritis. The prediction model is easy to use in clinical practice, for example by using a computer. By indicating which criteria are present and absent in a particular patient, the probabilities of the three forms of arthritis outcome can be simply obtained from the model. In the Leiden cohort the prediction model discriminates very well between the different forms of arthritis outcome.[35] The discriminative ability was expressed as the Area Under the Curve (AUC) of the Receiver Operator Characteristic (ROC). A ROC curve plots the relation between sensitivity on the Y-axis and (1 - specificity) on the X-axis, for different cut-off levels of test positivity. The area under the curve is a measure of the overall discriminative value of the model. A value of 0.5 means no discrimination at all, a value higher than 0.7 is acceptable and a value of 1 is perfect. The ROC AUC of the model for discrimination between self-limiting and persistent arthritis is 0.84 (SE 0.02) and for discrimination between erosive and non-erosive arthritis given persistence is 0.91 (SE 0.02). The discriminative ability of the 1987 ACR classification criteria is significantly lower, with ROC AUCs of 0.78 (SE 0.02) and 0.79 (SE 0.03), for self-limiting versus persistent arthritis and erosive versus non-erosive arthritis given persistence, respectively. It was concluded that the ability of the prediction model to discriminate between three forms of arthritis outcome is excellent and generates clinically relevant predictive values.[35] Before a prediction model is implemented into practice, adequate validation is required.[36] Validation means that the performance of a model is tested in a different patient cohort to the sample used to generate the model. A model can predict outcome well in the patients from which it was derived but may be unreliable elsewhere. At the moment the model is validated in different early arthritis cohorts.

Clinical value of anti-CCP antibodies

The sensitivity of the anti-CCP test for RA (the percentage of RA patients with positive test) is 60 to 88%, depending on the characteristics of the RA population.[26,29,33] The specificity of the test for RA (the percentage of non-RA patients with negative test) is very high: 96 to 99%, depending on the characteristics of the non-RA population.[26-33] However, patients and clinicians confronted with early arthritis need probabilities of the different forms of arthritis outcome to be able to choose management strategies, and it is impossible for the clinician to calculate these probabilities from the sensitivity and specificity of isolated tests. The prediction model for persistent (erosive) arthritis is an important and usable tool for prediction of arthritis outcome. The anti-CCP ELISA independently and significantly contributes to the performance of this prediction model.[35] The overall discriminative ability of the prediction model without anti-CCP test is significantly lower than that of the model with anti-CCP test: for persistent versus self-limiting arthritis: ROC AUC 0.82 (SE 0.02), for erosive versus non-erosive arthritis ROC AUC 0.90 (SE 0.02). Therefore, the anti-CCP test has added value in diagnostic and therapeutic decision-making in early arthritis, as indeed can also be concluded from the study by Vencovski *et al*.[32]

APOPTOSIS, AUTOIMMUNITY AND RA

The question arises why RA patients, and only RA patients, make these anti-CCP antibodies. Why are fibrin and probably other synovial proteins being citrullinated in the inflamed synovium during the disease? Such questions become even more intriguing when one realises that citrullination only occurs in certain specialised cell types (for example, myelin basic protein in glia cells) and in certain types of dying cells. Although the presence of apoptotic cells in synovial tissue is not obvious, it is possible that environmental factors (including pathogenic and inflammatory agents) induce abnormal cell death locally. It is not unlikely that during this process extravascular fibrin, and other synovial proteins, are targeted by activated PAD enzymes. We postulate that such modifications, taking place at local sites in the body, generate unique epitopes to which no effective tolerance exists.[37,38] In susceptible individuals a primary and specific immune response will then develop.

CONCLUSION

We have shown that among the many autoantibodies that can be detected in the serum of an RA patient, the autoantibodies directed to citrullinated antigens have a high potential for clinical use. Anti-CCP antibodies are very specific for the disease and can be detected early in the disease. In unselected early arthritis patients the test has added value in predicting persistent (erosive) arthritis. Further research is needed to show that citrullination of relevant self-proteins induces the production of autoantibodies. Such studies may also shed light on whether anticitrullinated protein antibodies have pathological effects or not.

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Collapse after moderate exercise

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A 37-year-old white woman collapsed at home after moderate physical exercise. Her partner called for emergency assistance. After about 15 minutes the paramedics arrived, at that time the patiënt was unresponsive with no palpable pulse. No electric activity of the heart was noted, and cardiopulmonary resuscitation (CPR) was started.

After 15 minutes of CPR, sinus rhythm with adequate circulation was restored. On arrival in the hospital she was unresponsive to auditive or tactile stimuli; her circulation was intact. A transthoracic echocardiography image is shown in *figure 1*. She died later of hypoxic brain damage.

WHAT IS YOUR DIAGNOSIS?

See page 416 for the answer to this photo quiz.

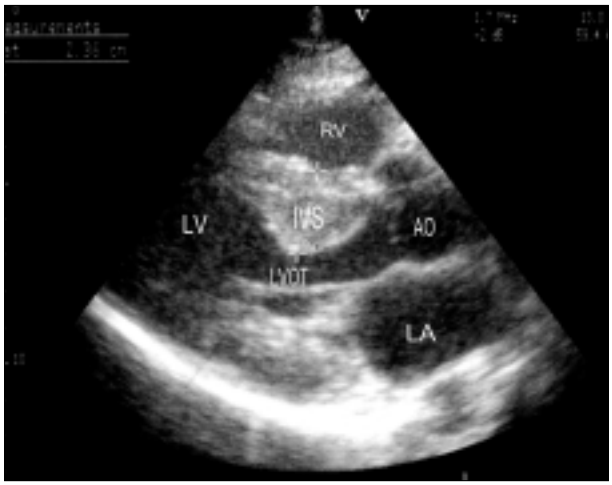

Figure 1
Two-dimensional echocardiogram (parasternal long-axis view)

RV = right ventricle, IVS = intraventricular septum, LV = left ventricle, AO = aorta, LVOT = left ventricular outflow tract, LA = left atrium.

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REVIEW

Pathogenesis of renal microvascular complications in diabetes mellitus

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INTRODUCTION

Microvascular disease is the main determinant in the development of late complications in diabetes mellitus. This is obvious for diabetic nephropathy[1,2] and retinopathy,[3,4] but changes in the microcirculation may also play an important role in the pathogenesis of diabetic neuropathy.[5,6] Although the pathogenesis of diabetic microangiopathy is incompletely understood, it is likely that it involves an interaction between metabolic and functional/haemodynamic factors. Together with genetic and environmental factors this results in the development of microvascular complications.

EPIDEMIOLOGY

Diabetic nephropathy is a major cause of morbidity and mortality in patients with type 1 as well as type 2 diabetes. Diabetic nephropathy is characterised by specific morphological changes including glomerular basement membrane thickening, mesangial expansion and glomerular and tubulo interstitial sclerosis. The clinical syndrome of diabetic nephropathy consists of proteinuria, hypertension and a progressive decrease in the glomerular filtration rate. Various studies reveal cumulative incidence rates for diabetic nephropathy of 20 to 40%,[7-9] but it is suggested that the incidence is declining.[10] Although the incidence of diabetic nephropathy among diabetic patients is decreasing, the prevalence of nephropathy among the population as a whole is dramatically increasing, predominantly due to improvement of hypertension and coronary heart disease, and due to an increase in prevalence of type 2 diabetes mellitus. The risk of diabetic nephropathy among type 2 diabetic patients with progression to end-stage renal disease is comparable with that in type 1 diabetes mellitus.[11,12] The peak incidence of diabetic nephropathy is between 10 and 17 years after the onset of diabetes.[8,13] Thereafter, the incidence of diabetic nephropathy declines rapidly. The first clinical manifestation of diabetic nephropathy is microalbuminuria, defined as a urinary albumin excretion rate of 20 to 200 μg/min. Microalbuminuria is associated with other microvascular complications as well as with cardiovascular disease suggesting some common pathophysiological mechanisms.[7,14]

PATHOPHYSIOLOGY

The pathophysiology of renal microvascular complications in diabetes mellitus consists of an intensive interplay of metabolic and functional/haemodynamic factors that underlies the structural changes of the microvasculature.

Structural changes

These structural abnormalities in diabetic glomerulopathy include an increase in basement membrane thickness, mesangial expansion with accumulation of extracellular matrix components (ECM) and glomerular fibrosis. There is an inverse correlation between heparan sulphate proteoglycan (HSPG) expression and mesangial expansion in diabetic glomerulopathy,[15] stressing the importance of dysregulation of ECM synthesis, which seems crucial for the development of renal microvascular complications. Furthermore, the extent of ECM accumulation correlates strongly with the degree of tubulointerstitial fibrosis and

of renal failure and proteinuria.[16-18] Tubulointerstitial injury is probably a major feature of disease progression.[19] Chronic interstitial injury usually follows the onset of glomerular proteinuria. Both structural lesions are probably linked via the increase in glomerular permeability and ultrafiltration of bioactive (growth) factors which can be held responsible for inducing and/or aggravating ECM production and renal fibrosis.

Metabolic pathways

Several factors such as high glucose, intracellular polyols, non-enzymatic glycation products, hexosamines and vasoactive hormones are held responsible for the changes in regulation of the biosynthesis of matrix components composition and accumulation.[20] They stimulate the synthesis and release of growth factors and cytokines from resident renal cells, inducing cell proliferation and hypertrophy, as well as the production of extracellular matrix proteins.[21] Of these the profibrotic cytokine, transforming growth factor-β (TGF-β), has emerged as a key factor in the development of structural abnormalities in diabetic nephropathy.[22] *In vitro* experiments showed that glomerular mesangial cells, epithelial cells and interstitial fibroblasts increased their TGF-β expression when exposed to high glucose.[23,24] Also, *in vivo*, the expression of renal TGF-β is increased in experimental as well as in human diabetes.[25,26] In animal models, neutralising TGF-β antibodies prevented the increase in extracellular matrix components and the increase in mRNA encoding for type IV collagen α1 and fibronectin. Furthermore, anti-TGF-β almost completely prevented the fall in creatinine clearance in diabetic *db/db* mice.[27]

Except for ECM accumulation, it is suggested that changes in the heparan sulphate chains of HSPG in glomerular basement membranes and ECM play a role in diabetic renal disease. A decrease in heparan sulphate, the anionic side chain of HSPG, induces proteinuria, stressing the importance of heparan sulphate for the permselectivity of the glomerular basement membrane.[28] It has been suggested that proteinuria itself plays a pathogenic role in diabetic nephropathy.[29] However, in contrast to the prevention of the decrease in glomerular filtration rate, neutralising TGF-β antibodies did not prevent proteinuria in diabetic mice.[27] This could implicate that the detrimental effects of proteinuria on renal function are mediated via TGB-β or that proteinuria is just a consequence of permselectivity changes.

Nevertheless, heparan sulphate also determines the local concentration, compartmentalisation, stability and activity of certain growth factors and proteases, thus controlling ECM expansion.[30] Heparan sulphate is probably directly involved in the inhibition of TGF-β overexpression[31] and regulates the expression of decorin, an extracellular matrix protein that inactivates TGF-β.[32]

Hyperglycaemia

The link between ECM accumulation via TGF-β and other profibrotic cytokines and diabetes is met by metabolic changes characteristic for the diabetes state per se. The metabolic hypothesis suggests that microvascular complications develop as a direct consequence of hyperglycaemia. Several small, prospective, randomised intervention studies and the Diabetes Control and Complications Trial (DCCT)[33] have definitely proven that improved metabolic control achieving near-normoglycaemia can reduce the incidence of diabetic nephropathy. These studies revealed duration and severity of hyperglycaemia as major risk factors for the development of diabetic microvascular complications. Complete normalisation of blood glucose after pancreas transplantation even shows a regression of structural renal changes.[34]

The mechanisms by which hyperglycaemia gives rise to microvascular complications have slowly been unravelled in the last years, supported by a large amount of experimental as well as clinical data.

Advanced glycation end products

Advanced glycation end products (AGEs), which are formed by non-enzymatic glycation of proteins, accumulate in renal glomeruli.[35] Recent research has shown that AGE precursors (dicarbonyls, such as methylglyoxal) are formed intracellularly from intracellular hyperglycaemia. These precursors can react with amino groups of intracellular and extracellular proteins to form AGEs. AGEs are capable of inducing increased vascular permeability, enhancing protein and lipoprotein deposition, inactivating nitric oxide and promoting matrix protein synthesis and glomerular sclerosis.[36] This last mechanism is probably mediated by TGF-β.[37,38]

The clinical importance of AGEs in diabetic nephropathy is stressed by the role of AGE formation inhibitors such as aminoguanidine and ALT-946 and the so-called AGE cross-link 'breakers' (phenacylthiazolium bromide; ALT-711) for treatment or prevention of diabetic (renal) complications.[39,40]

Protein kinase C

An increase in intracellular glucose induces *de novo* synthesis of diacyl glycerol (DAG)[41] which activates protein kinase C (PKC). PKC is capable of phosphorylating a number of cellular proteins. Increased PKC activity modulates gene expression in mesangial cells, inducing extracellular matrix protein synthesis, especially of type IV collagen and fibronectin, which is mediated by TGF-β.[22,42] Furthermore, PKC activation is linked to mitogen-activated protein kinase (MAPK), which is important in the intracellular signal transduction processes leading to cell proliferation and hypertrophy.[43,44] Activation of PKC increases production of vasodilatory prostanoids leading to hyperfiltration.[45] Animal studies showed that blockade

of PKC by means of LY333531 reversed renal hyperfiltration and increased glomerular albumin permeability.[46] Treatment with a PKC inhibitor showed a reduction in urinary albumin excretion rates and prevented mesangial expansion observed in diabetic *db/db* mice, possibly through attenuation of glomerular expression of TGF-β.[47]

Polyol pathway

Hyperglycaemia induces an increased flux through the polyol pathway. Originally it was thought that intracellular formation and accumulation of sorbitol, mediated by aldose reductase, leads to increased intracellular osmolality and swelling of cells. However, just recently it has been shown that decreased levels of reduced glutathione (GSH), as a result of the reduction from glucose to sorbitol,[48] are responsible for the deleterious consequences by increasing intracellular oxidative stress (see below). Clinical trials suggested the potential usefulness of aldose-reductase inhibitors in preventing the progression of incipient diabetic nephropathy in patients with type 2 diabetes mellitus.[49]

Hexosamine pathway

In diabetes an increased flux of intracellular glucose through the hexosamine pathway results in increased N-acetylglucosamine (GlcNAc), by conversion from fructose-6-phosphate by the enzyme GFAT. Most probably, modification of transcription factors such as Sp1 by GlcNAc will lead to transcription of the gene for TGF-β.[49] Furthermore, GlcNAc modifies many other intracellular proteins such as eNOS activity[49] that may contribute to the pathogenesis of renal diabetic complications.

Reactive oxygen species as a common pathway

Recently, Nishikawa *et al.*[50] and Du *et al.*[51] demonstrated that the formation of AGEs, activation of PKC, and activation of the polyol pathway as well as the hexosamine pathway, are mediated by the production of reactive oxygen species (ROS). They showed that elevated (intracellular) glucose levels increase the production of ROS in mitochondria. This over-production of ROS was prevented by manganese superoxide dismutase or by an uncoupler of oxidative phosphorylation, by uncoupling protein-1, and completely prevented intracellular formation of AGEs, activation of protein kinase C, increase in polyol pathway flux and hexosamine pathway activation. They concluded that ROS production is a common pathway in the initiation of high glucose-mediated stimulation of the aforementioned pathways.
It is hypothesised that an excess of ROS inhibits GADPH (glyceraldehyde-3-phosphate dehydrogenase), a glycolytic key enzyme promoting shunting of upstream glucose metabolites into the aforementioned pathways.

Functional/haemodynamic pathway

Long-lasting poor metabolic control does not necessarily lead to diabetic microvascular disease. This means that the apparent protection of diabetic patients for nephropathy cannot solely be explained on the basis of better metabolic control. The haemodynamic hypothesis implies that due to haemodynamic alterations in blood flow and pressure, structural changes are provoked, which will result in the development of microvascular complications.

Flow/pressure (haemodynamic hypothesis)

Capillary hyperperfusion precedes the onset of diabetic renal microangiopathy.[2] This observation has led to the hypothesis that changes in systemic or local haemodynamics contribute to the development of diabetic nephropathy.[52]
Micropuncture studies revealed a range of haemodynamic alterations in diabetes: increased intraglomerular pressure, increased single nephron GFR and preferential afferent compared with efferent arteriolar vasodilation. These renal haemodynamic changes may be related to vasoactive hormones such as angiotensin II, endothelin, nitric oxide, locally active prostaglandins and kinins, and atrial natriuretic peptide. Furthermore, hyperglycaemia, glucagon, insulin, insulin-like growth factor and reduced sympathetic nerve activity may be involved in diabetic microvascular haemodynamic changes. These haemodynamic changes cause injury to the vascular wall, resulting in increased permeability, intima fibrosis, and vascular smooth muscle cell proliferation.[53]
Therapy aimed at reversing glomerular hyperfiltration, by controlling glucose concentration early in the course of the disease, dietary protein restriction, and antihypertensive therapy, may slow the rate of progression of the renal disease. Many pharmacological substances are currently being developed which block the effect of vasoconstrictory hormones or reduce the degradation of vasodilating hormones. Because most of the enzymes involved in production and degradation of these vasoactive hormones have considerable homology, substances are being developed which interact with more than one of these systems.

Renin-angiotensin system (RAS)

The therapeutic effects of angiotensin-converting enzyme (ACE) inhibitors and AT_1-receptor antagonists in decreasing the progression of microalbuminuria or macroalbuminuria stresses the importance of the renin-angiotensin system (RAS).[54-58] The decrease in progression of diabetic nephropathy by ACE inhibitors and AT_1-receptor antagonists was originally attributed to their ability to control systemic and intraglomerular hypertension. However, despite comparable reductions in systemic blood pressure, endothelin-receptor blockade was not as renoprotective as an ACE inhibitor, at least in rats.[59] Indeed, the aforementioned studies showed that effective blockade of angiotensin II action had favourable renoprotective effects that go beyond the blood pressure lowering effects of these drugs. Thus, it is likely

that some of these favourable effects might be related to the non-haemodynamic effects of angiotensin II. Angiotensin II induces smooth muscle cell growth, hypertrophy and proliferation of glomerular cells and stimulates the synthesis of ECM components, collagen and fibronectin. The angiotensin II-induced smooth muscle cell growth and the hypertrophic and fibrogenic responses are mediated by TGF-β.[60] Neutralising anti-TGF-β antibodies are able to prevent angiotensin II-stimulated production of ECM in *in vitro* studies with mesangial cells.[61] Furthermore, angiotensin II activates PKC in glomerular cells through AT_1-receptor stimulation.

Endothelial dysfunction

The normal endothelium has important regulating properties for vascular tone and is intimately involved in the regulation of vascular and renal permeability. It regulates the composition of ECM and the proliferation of smooth muscle and mesangial cells.[62] Therefore, endothelial dysfunction has been implicated in the pathogenesis of diabetic vascular disease. In non-complicated type 1 diabetic patients acetylcholine-induced endothelial-dependent vasodilatation is intact.[63] In contrast, diabetic microalbuminuria reflects widespread endothelial dysfunction. An increase in von Willebrand factor (vWF), a component of the endothelial cell membrane and a marker of endothelial damage, precedes the occurrence of microalbuminuria.[64] The mechanisms by which diabetes causes impaired endothelial function are largely unknown. In view of the haemodynamic changes in diabetes a biphasic process is proposed. As a vasodilator, nitric oxide (NO) is a candidate for mediating the increases in blood flow and capillary permeability that are observed in the early phase of diabetes. Indeed, an increased basal endogenous NO production accounts for the renal hyperfiltration in diabetic rats, whereas in the kidney there seems to be an enhanced nitric oxide production indicated by an increased urinary nitrate and nitrite concentration.[65,66] The cause of the increased renal nitric oxide production has not yet been elucidated. There are reports of an increased expression of inducible nitric oxide synthase (iNOS),[67] but animal studies showed that l-imino-ethyl-lysine, a specific inhibitor of iNOS, was unable to reduce the glomerular hyperfiltration.[68] Furthermore NO was not increased in normoalbuminuric type 1 diabetic patients using the isolated forearm technique.[63]

Deficiency of NO in the vascular tree is a duration-dependent process. Therefore, late in the course of diabetes, damaged endothelial cells may loose the ability to increase NO synthesis, thus favouring a proliferative and thrombogenic milieu. It is suggested that endothelial dysfunction could be the result of hyperglycaemia-induced formation of free radicals, which inactivate NO. *In vitro*, the bioavailability of NO is reduced by AGEs, which quench NO.[36] Also, hyperglycaemia interferes with the production of cyclic guanylate monophosphate (cGMP), the second messenger of NO. Hyperglycaemia is also capable of activating protein kinase C that inhibits endothelial nitric oxide synthase (eNOS).[42]

Of interest, it was recently suggested that activation of poly(ADP-ribose) polymerase (PARP) is an important factor in the pathogenesis of endothelial dysfunction in diabetes. Inhibition of PARP by a novel PARP-inhibitor PJ34 maintained normal vascular responsiveness, despite persisting hyperglycaemia in diabetic mice.[69]

Genetic influences

In contrast to diabetic retinopathy and neuropathy, which develop in the majority of diabetic patients, only 30 to 40% of type 1 diabetic patients are at risk of developing diabetic nephropathy. In view of the observed familial clustering of diabetic nephropathy, a genetic predisposition to diabetic nephropathy has been assumed. From epidemiological studies evidence appears about genetic influences on the development of microvascular complications.[70-72] The genetic susceptibility may also explain the marked differences in incidence of microvascular complications between various races.[73]

A genetic predisposition to and a parental history of hypertension are supposed to be risk factors for the development of diabetic nephropathy.[74] The likelihood of developing diabetic nephropathy was increased in patients with an elevated sodium-lithium countertransport activity, a marker of the genetic predisposition to essential hypertension.[75]

As both hypertension and the development of diabetic nephropathy seem to be genetically determined, it is tempting to search for combined genetic markers. Genes involved in the RAS are promising candidates as the RAS plays a central role in both blood pressure regulation and renal function. Association studies linking these polymorphisms with the development of diabetic nephropathy reveal conflicting results. Many reports on ACE polymorphisms suggest a contribution of the DD polymorphism in the development of diabetic nephropathy,[76] although a recent thorough review by Kunz *et al.*[77] failed to confirm the suggested association due to methodological limitations in the original studies.

CONCLUSIONS

The pathophysiology of renal diabetic microvascular complications is now slowly being unravelled. Metabolic and haemodynamic changes interfere and TGF-β seems to play a central role in this process. There is also strong evidence that hereditary factors are essential in the

development of microvascular complications. This suggests that the development of diabetic microvascular complications is a multifactorial process in which different mechanisms are likely to operate. Elucidating the pathophysiology of microvascular complications is important for the development of appropriate prevention and treatment of these complications.

NOTE

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Single-centre experience with tunnelled central venous catheters in 150 cancer patients

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ABSTRACT

Background: Tunnelled venous catheters improve venous access in cancer patients, but are associated with complications. We retrospectively analysed the outcome of Hickman catheter and Port-A-Cath® (PAC) insertion in cancer patients from a department of medical oncology and compared these results with the literature.

Methods: The files of patients in whom insertion of a Hickman or PAC was planned in the period March 1992 to August 1999 were analysed.

Results: In total, 150 files were evaluated. In 149 patients, 128 Hickman catheters and 38 PACs were inserted successfully. Complications occurred in 44.6% of the catheters inserted successfully. Infection (24.1%) and thrombosis (7.2%) were observed most frequently. In 66.7% of patients with thrombosis, the catheter tip was positioned incorrectly. Removal for catheter-related complications occurred in 47.7%. Of 146 catheter insertions for which the name of the responsible (resident) surgeon could be traced, 48 different names were identified.

Conclusions: In comparison with other studies, we found a high incidence of infections and a high removal rate for catheter-related complications. We confirmed the relationship between thrombosis and an inadequate position of the catheter tip. The level of experience of the (resident) surgeon performing the catheter insertion may have played a role in the high complication rate.

INTRODUCTION

The systemic treatment of malignant tumours often requires prolonged venous access. Since the introduction of the tunnelled, partly external central venous catheter by Broviac *et al.*,[1] its modification by Hickman *et al.*[2] and the introduction of the totally implantable venous access device Port-A-Cath® (PAC), the maintenance of adequate venous access in the treatment of cancer patients has improved substantially. However, insertion of a Hickman catheter or PAC is associated with complications. The most important are infection, thrombosis and pneumothorax, which occur for the Hickman in 13 to 14.9%,[3,4] 3.8 to 10%[3,4] and 1.6%,[3] respectively and for PAC in 2.2 to 9%,[4-8] <1 to 16%[4-9] and <1 to 1.5%,[5,6,8] respectively. In this retrospective study we analysed the results of Hickman and PAC insertion in cancer patients from a department of medical oncology and compare these results with previously published studies.

PATIENTS AND METHODS

The files of all patients from our Department of Medical Oncology who participated in clinical studies and received a Hickman or PAC catheter in the period March 1992 to August 1999 were acquired. As to the choice for either Hickman or PAC, a PAC was preferred when long-term use (i.e. >3-4 months) was expected. Catheter insertion in patients outside these trials was negligible. The following data were collected: age, primary tumour, indication for inserting the catheter, type of catheter, time to removal of catheter, reason for removal, time to occurrence of complication and type of complication.

A diagnosis of catheter infection was made in the case of high clinical suspicion (i.e. local pain, redness) in combination with fever, necessitating removal of the catheter. In all patients in whom this diagnosis was made, bacterial cultures of the catheter tip and/or peripheral blood were obtained. A catheter infection occurring within two weeks after insertion was considered to be related to the surgical procedure. During this period the white blood cell count was still normal in all our patients.
A diagnosis of catheter-related thrombosis was suspected in case of symptoms such as impaired catheter flow, swelling or oedema of the arm or the development of collateral veins in the shoulder/chest area. This diagnosis had to be confirmed by ultrasound or phlebography. The presence of a pneumothorax and the position of the catheter was checked by means of chest X-rays directly after and on the morning of the day after catheter insertion. Accidental removal of the catheter was defined as accidental translocation of the catheter preventing its use.

RESULTS

The files of 150 patients were evaluated. All patients were treated for advanced solid tumours, mainly breast cancer (30%) and melanoma (27%). Median age was 48 years (range 19-75 years). All catheters were inserted under local anaesthesia by surgeons or residents of the Department of Surgery. Catheter insertion failed in 11 patients leading to pneumothorax in one. In ten of these patients the catheter was successfully inserted during a subsequent session. In one patient further attempts were abandoned. A total of 166 catheters were successfully inserted in 149 patients: 128 Hickman catheters (3 single, 88 double and 37 triple lumen catheters) in 114 patients, and 38 PACs in 37 patients. Two patients received a Hickman and subsequently a PAC. All but two catheters were used for high-dose chemotherapy plus peripheral stem cell infusions, continuous 5-fluorouracil infusion or interleukin-2 (Il-2). The reason for intentional removal of catheters could be traced for 113 Hickman catheters and 19 PACs. End of treatment or death of the patient was a reason for removal in 53/113 (46.9%) of the Hickman catheters and 16/19 (84.2%) PACs, respectively. Complication necessitated the removal of 60/113 (53.1%) Hickman and 3/19 (15.8%) PACs. Median time to removal was 54 days (range 1-174) for the Hickman and 142 days (range 12-372) for the PAC. The incidence of complications was 66/128 (51.6%) for successfully placed Hickman catheters and 8/38 (21.1%) for PACs, respectively. Infection, thrombosis, pneumothorax, accidental removal of the catheter, malpositioning of the catheter and pain were observed (*table 1*). The median time to the occurrence of complications was 34.5 days (range 0-178) (*table 2*).
Infection necessitating removal of the catheter occurred in 40/166 (24.1%) catheters: 38/128 (29.7%) Hickman catheters and 2/38 (5.3%) PACs. In blood cultures *Staphylococcus aureus* was detected most frequently (46%), followed by *Staphylococcus epidermidis* (25%). The median time to infection was 38 days (range 8-141). Within two weeks after catheter insertion 22.5% of the infections occurred. The incidence of infections in patients who were given a Hickman catheter for Il-2 or peripheral stem-cell transfusion was 31.7%. Hickman catheters placed for other indications resulted in infections in 20.8% of the cases.

Table 1
Complications after successful placement of tunnelled central venous catheters

COMPLICATION	TOTAL N=166 NUMBER (%)	HICKMAN N=128 NUMBER (%)	PAC N=38 NUMBER (%)
Infection with removal of catheter	40 (24.1)	38 (29.7)	2 (5.3)
- Positive local cultures*	12 (7.2)	11 (8.6)	1 (2.6)
- Positive blood cultures**	22 (12.4)	21 (16.4)	1 (2.6)
- Negative cultures	6 (3.6)	6 (4.7)	0
Thrombosis	12 (7.2)	9 (7.0)	3 (7.9)
Pneumothorax	4 (2.4)	4 (3.1)	0
Accidental removal	11 (6.6)	11 (8.6)	0
Misplacement	6 (3.6)	4 (3.1)	2 (5.3)
- With removal of catheter	3 (1.8)	3 (2.3)	0
- With revision of catheter	3 (1.8)	1 (0.8)	2 (5.3)
Pain	1 (0.6)	0	1 (2.6)
Total number of complications	74 (44.6)	66 (51.6)	8 (21.1)

** Catheter tip of pocket PAC, ** peripheral blood or blood from venous catheter.*

Table 2
Time to occurrence of catheter-related complications in days

COMPLICATION	TOTAL MEDIAN (RANGE)	HICKMAN MEDIAN (RANGE)	PAC MEDIAN (RANGE)
Infection with removal of catheter[1]			
- Positive local cultures*	37.0 (19.0-95.0)	37.5 (14.0-95.0)	10.0
- Positive blood cultures**	36.5 (8.0-141.0)	33.0 (8.0-105.0)	141.0
- Negative cultures	61.5 (30.0-98.0)	61.5 (30.0-98.0)	
Thrombosis	40.0 (8.0-178.0)	40.0 (8.0-145.0)	176.0 (23.0-178.0)
Pneumothorax	1.0 (0-2.0)	1.0 (0.0-2.0)	-
Accidental removal	24.0 (2.0-89.0)	24.0 (2.0-89.0)	-
Misplacement			
- With revision of catheter	2.0 (1.0-36.0)	1.0 (1.0-1.0)	19.0 (2.0-26.0)

[1] *The time indicated is time to occurrence of symptoms of fever and/or local inflammation, * catheter tip of pocket PAC, ** peripheral blood or blood from venous catheter.*

Thrombosis was diagnosed in 12 (7.2%) cases, 9/128 (7%) in Hickman catheters and 3/38 (7.9%) in PACs. All patients were treated with anticoagulants and in case of a PAC the catheter was left in situ. In 8/12 (66.7%) patients with thrombosis, the chest X-ray taken after insertion showed an incorrect position of the catheter tip with all of the distal part of the catheter in a horizontal position.
Pneumothorax was not a reason for removal of catheters and was treated conservatively in half of the cases.
Accidental removal of the catheter was observed in 11/128 (8.6%) of the Hickman catheters. Six of these removals occurred after the three-week period, which is supposed to be sufficient for adequate fixation of the catheter to the surrounding tissue. No accidental removals were observed for PACs. In 4/128 (3.1%) of the Hickman catheters and 2/38 (5.3%) of the PACs, the catheter tip was inadequately positioned leading to removal of three Hickmans.
Shoulder and neck pain occurred in one patient with a PAC necessitating its removal which lead to improvement. The adequate position of the PAC was confirmed radiologically.
Of 146 catheters inserted the name of the responsible (resident) surgeon could be traced. Forty-eight different names were identified. The maximum number of catheters inserted by one (resident) surgeon was 12. In 20 cases the responsible (resident) surgeon could not be traced.

DISCUSSION

Venous catheters like the Hickman and PAC are often used in cancer patients. However, the use of these catheters is not without complications. Previously, we reported the incidence of spontaneous rupture of central venous catheters in relation to their position at the thoracic outlet.[10] This complication did not occur in the present series.

Other studies report a complication rate between 32.1 to 47.6% for Hickman catheters[3,4] and 12.8 to 53.0% for PACs.[4-8] In our study Hickman catheters show a higher total incidence of complications (51.6%), due to a larger number of infections (29.7%) and accidental removals (8.6%). In recent studies these complications were observed in 13.0 to 14.9% and 0.8%, respectively.[3,4]
The high incidence of infections in our population can be partly explained by the increased risk for infection associated with treatment with interleukin-2[11] due to a reduced function of granulocytes[12,13] or with prolonged granulocytopenia after high-dose chemotherapy. However, 22.5% of the total incidence of infections occurred within two weeks after placement of the catheter. During this period a severe granulocytopenia had not yet been observed in our patients. Therefore, in these cases a relation between infection and the insertion procedure is suspected.
The type of dressing applied to the catheter site has also been suggested to influence the incidence of catheter-related infection. The application of occlusive plastic dressings instead of gauze dressings to the insertion site would increase the risk of infection by producing a warm, moist environment that facilitates bacterial growth. This hypothesis was confirmed in a large meta-analysis.[14] However, these conclusions may not be applicable to the more recently introduced non-occlusive transparent dressings.[15] In our department's protocol it is recommended to use sterile gauzes instead of non-occlusive transparent dressings when the insertion site is moist or the patient is perspiring heavily.
There is conflicting evidence on the value of prophylactic antibiotics given prior to insertion of central venous catheters.[3] Although studies have shown a reduced risk of infection after antibiotic prophylaxis, this benefit may particularly apply for specific patient groups, such as patients who are neutropenic at the time of infection.[16] The patients in our series did not receive prophylactic antibiotics.

In our series only clinically significant catheter-related infection necessitating removal of the catheter has been scored. Since the central venous catheter is often the only access for administration of chemotherapy, its preservation in situ is of great importance. By administration of systemic antibiotics or an 'antibiotic lock' control of infection could be achieved while leaving the catheter in situ.[17] However, when fever is present as a sign of systemic infection, we recommend prompt removal of the catheter in this patient category which may develop granulocytopenia or reduced function of granulocytes.

S. aureus and *S. epidermidis* were the micro-organisms cultured most frequently in our series, which is confirmed in other studies.[3,4,7,8] Although coagulase-negative staphylococci are becoming more frequent than *S. aureus* as the cause of catheter-related infection,[18] in some series, like ours, *S. aureus* is still the major cause.[19]

The accidental removal of catheters was restricted to Hickman catheters and is likely to be caused by the presence of an external part of the catheter, which is prone to accidental manipulation. The observation that more than half of the catheters were accidentally removed after the three-week period, which is supposed to be sufficient for adequate fixation of the catheter to the surrounding tissue, could be explained by the fact that all patients received cytotoxic or immunosuppressive therapy. This might have caused delayed granulation around the catheter tip, delaying catheter fixation. Manipulation of the catheter should therefore be reduced as much as possible for at least three weeks after insertion and should be performed with great care.

Our reported incidence of thrombosis is comparable with previous studies.[3-9] We,[20] as well as others,[4,8,9] have described a correlation between an inadequate insertion depth of the catheter tip and thrombosis of the subclavian vein. When the catheter is not inserted to an adequate depth and remains in the brachiocephalic vein, the chest X-ray shows a horizontal course of the distal part of the catheter instead of a vertical course in the superior caval vein towards the right atrium. This inadequate position causes an increased contact of the catheter tip with the vessel wall, possibly causing endothelial damage. This endothelial damage could explain the increased incidence of thrombosis. In our present study we found that the catheter was inadequately positioned in 66.7% of patients with a thrombosis. In one study the correction of an inadequate catheter position reduced the incidence of thrombosis from 4.3 to 1.0%.[9] Thrombosis occurred much later in patients with PACs compared with Hickman catheters (*table 2*). However, these results should be interpreted with caution because the incidence of thrombosis in patients with a PAC was quite low and the period of insertion for PAC was much longer compared with the Hickman catheter (median of 142 versus 54 days, respectively), implicating a much more prolonged period at risk for this event.

The incidence of pneumothorax in our series is higher compared with a previous large study,[3] 3.1 versus 1.6%, respectively. Several patients were diagnosed with pneumothorax the day after insertion while the chest X-ray directly after insertion was normal. This underscores the need of delayed chest X-ray control.

In our series catheter-related pain occurred only once with a PAC and not with Hickman catheters. In a large prospective study late-onset shoulder pain was observed in 4.8% of the patients with Hickman catheters.[21] Shoulder pain is associated with an increased risk of thrombosis and infection and may in some cases be due to extravasation of chemotherapy along a fibrin sleeve around the catheter.[22] In all patients complaining of pain, radiological and microbiological investigations are advocated to exclude thrombosis and infection.[21]

Several studies have confirmed the relation between experience of the surgeon and the incidence of complications.[3,9,21,23] Therefore, the low number of catheters that were inserted per surgeon (mostly residents) may be a causative factor in the high complication rate in our series. In training hospitals, as our institute, a strict supervision of an experienced surgeon is therefore recommended.

CONCLUSION

In conclusion, in comparison with the literature, we found a comparable total incidence of complications for PACs, but a higher incidence for Hickman catheters. For the Hickman catheter this was explained by a higher incidence of infections and pneumothorax in our series. We consider the high rate of catheter removal as a result of complications in our series not acceptable. The inexperience of surgeons in training who were responsible for the procedure of catheter insertion may have played a role in this. These data have resulted in a more stringent supervision of this procedure by experienced surgeons in our hospital.

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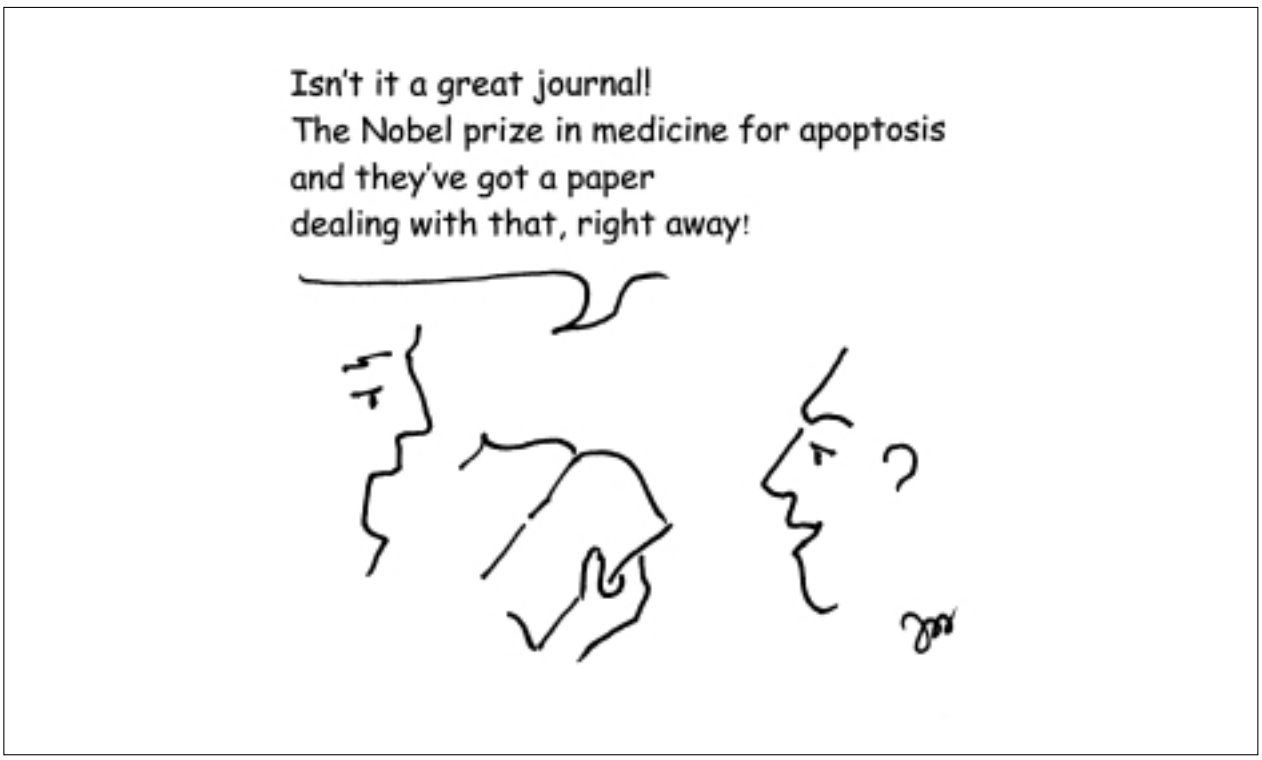

Koolen, et al. Single-centre experience with tunnelled central venous catheters in 150 cancer patients.

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Treatment of primary Sjögren's syndrome with D-penicillamine: a pilot study

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ABSTRACT

Background: Up to now no satisfying systemic treatment is available for patients with primary Sjögren's syndrome.

Methods: In a prospective, open study we investigated the effect of D-penicillamine (first three months 250 mg/day, next three months 500 mg/day) on clinical and immunological parameters in 19 patients with primary Sjögren's syndrome and a mean disease duration of 3.8 years.

Results: Eight patients had to stop treatment mainly due to severe (reversible) loss of taste. Clinically, a statistically significant increase in basal salivary flow was observed after three months ($p<0.05$). In addition, improvement was noted in the Schirmer test and stimulated parotid salivary flow after six months, but these differences were not statistically significant.

Laboratory values showed a decrease in ESR ($p<0.05$) and levels of IgA and IgM (both $p<0.02$) after six months, a decrease in levels of IgA-Rf and IgM-Rf after three months (both $p<0.05$), and an increase in haemoglobin level ($p<0.05$).

Conclusion: From this pilot study we conclude that the treatment of primary Sjögren's syndrome with D-penicillamine has only marginal beneficial effects. Together with its clear side effects this means that D-penicillamine is unsuitable for this indication.

INTRODUCTION

Primary Sjögren's syndrome (PSS) is a systemic autoimmune disease of the exocrine glands, in particular the lacrimal and salivary glands, frequently accompanied by extraglandular symptoms. Polyclonal B-cell activation with production of different autoantibodies such as antinuclear antibodies and rheumatoid factors (Rf) are characteristic serological findings.

Symptoms due to lacrimal and salivary gland involvement can be treated locally. Studies on treatment of PSS with disease-modifying drugs are relatively scarce. A significant reduction in levels of immunoglobulin G and M (IgG and IgM) was seen during treatment with hydroxychloroquine.[1] However, no beneficial effect on clinical symptoms was apparent.[1] Cyclosporin A subjectively improved xerostomia[2] and methotrexate subjectively improved both xerostomia and xerophtalmia as well as arthralgia[3] but no objective effect on clinical symptoms nor an effect on serological parameters was observed. Recently, azathioprine did not show any effect on clinical symptoms or serological findings; in particular, no decrease in levels of immunoglobulins was observed.[4]

D-penicillamine (D-pen) is a disease-modifying drug that has been proven efficacious in the treatment of rheumatoid arthritis (RA). Its mechanisms of action are largely unknown. *In vitro*, D-pen inhibits lymphoblastic transformation induced by polyclonal mitogens and decreases T-cell-dependent production of immunoglobulins by lymphocytes stimulated with Pokeweed mitogen. This inhibitory action is exercised on T lymphocytes. D-pen does not influence B lymphocytes directly.[5,6]

Several publications indicate that treatment with D-pen in

RA lowers the levels of rheumatoid factors, in particular IgM-Rf.[5,7-10] However, no correlation was demonstrated between the decrease in levels of IgM-Rf and the decrease in ESR or clinical disease activity.[7]
In many patients with PSS rheumatoid factors are detectable, frequently at a high concentration. The sublabial and parotid salivary glands have been reported to harbour increased amounts of IgG- and IgM-producing plasma cells.[11,12] It has been demonstrated that rheumatoid factors, antinuclear antibodies and other immunoglobulins are produced locally in the salivary glands of these patients.[13,14] Recently, levels of IgA-Rf were reported to correlate inversely with stimulated parotid flow rate.[15] Although the pathogenic significance of the local and systemic B-cell response is questionable, we may hypothesise from the foregoing data that treatment with D-pen might be beneficial in PSS.
In a Medline search (from 1966 onwards) only one report was found concerning treatment of PSS with D-pen.[16] In all four patients a subjective improvement was seen combined with a lowering of ESR and levels of rheumatoid factors, antinuclear antibodies and gammaglobulin fraction.
The primary objective of this study was to evaluate, in an open uncontrolled study, the clinical and immunological effects of D-pen in the treatment of PSS. Moreover, we evaluated the occurrence of side effects of D-pen treatment in PSS.

PATIENTS AND METHODS

All patients visiting the rheumatology outpatient clinic of our hospital and fulfilling the criteria for the diagnosis of primary Sjögren's syndrome[17] were asked to participate in the study. Exclusion criteria were age <18 years, treatment with cytostatics or disease-modifying drugs during the last six months, use of prednisolone during the last three months, the co-existence of other systemic autoimmune diseases such as RA, SLE and systemic sclerosis, proteinuria >1 g/day, leucopenia $<2.0.10^9$/l, thrombocytopenia $<100.10^9$/l, pregnancy or the presence of childbearing potential without adequate contraception. All subjects gave written informed consent.
The patients started at a dosage of 250 mg D-pen a day, taken half an hour before or two hours after a meal. After three months the dosage was increased to 500 mg a day. At baseline the demographic data and medication used were recorded. In addition, a complete history, physical examination and routine laboratory examinations including whole blood count, renal and hepatic function, and urinalysis were performed. For safety purposes the subjects were seen at three to six weeks intervals to screen for clinical side effects and for measurements of complete blood count and urinalysis. Comedication, if used, was not changed during the study.
The following clinical parameters were assessed at baseline, after three months and after six months: average value of Schirmer test without analgesia (mm/5 minutes; twice on both eyes), average value of tear break-up time (seconds; twice on both eyes), basal salivary flow (ml/15 minutes), average value of stimulated parotid (both sides) salivary flow (ml/10 minutes), dry eyes (visual analogue scale (VAS), 0-10 points), feeling of sand in the eyes (VAS, 0-10 points) and dry mouth (VAS, 0-10 points). The primary endpoints were Schirmer test and stimulated parotid salivary flow: an increase ≥25% was considered clinically significant.
The following laboratory parameters were assessed: ESR (mm/first hour), haemoglobin (mmol/l), serum IgA (0.5-3.7), IgG (8-17), IgM (0.4-2.3; g/l; turbidimetric method), IgA-Rf and IgM-Rf (IU/ml; as described previously)[18] and β_2-microglobulin (normal value <1.9 mg/l; radioimmunoassay, Pharmacia). All these laboratory measurements were performed serially from stored samples except for the ESR and haemoglobin.

STATISTICS

Spearman's rank-sum test was calculated for detecting a possible correlation between the different baseline study parameters. For comparison of groups, chi-square analysis was applied for discrete variables and Kruskal-Wallis ANOVA analysis for continuous variables. Changes of variables after intervention were evaluated with Wilcoxon's test for paired observations. A p value of ≤0.05 was considered significant.

RESULTS

Nineteen patients fulfilling the criteria for primary Sjögren's syndrome[17] were initially included. In *table 1* data are given on basic characteristics of these 19 subjects. Four patients had to stop with D-pen (250 mg/day) within three months, three because of severe loss of taste and one because of severe dermatitis and cheilitis. All these side effects were completely reversible after stopping the study medication. Fifteen patients completed at least three months treatment. These 15 patients are discussed in further detail. The group of 15 'completers' did not differ from the four 'non-completers' with respect to the characteristics from *table 1* except for a higher frequency of extraglandular symptoms in the last group ($p<0.05$). During the next three months, following increase of the dosage to 500 mg/day, three other patients had to stop D-pen because of severe side effects due to severe loss of taste, nausea and anorexia, and malaise and diplopia, respectively. All these side

Table 1

Characteristics (mean, range) of the 19 initially included patients with primary Sjögren's syndrome[17]

Age (years)	62.4 (34-79)
Sex (male/female)	4/15
Duration of established disease (years)	3.8 (0.5-11)
Anti-SSA and/or anti-SSB	12
Parotitis, past or present	4
Extraglandular manifestations*	6
Focus score ≥1 on minor salivary gland biopsy	12 (out of 16)

* *Polyneuropathy (2), cutaneous vasculitis in the past (1), optic neuritis in the past (1), distal renal tubular acidosis and encephalopathy possibly due to Sjögren's syndrome (1).*

effects were reversible after the study medication had been stopped. In one other patient the protocol was violated after three months as prednisolone had to be given because of severe polyarthritis. So, 11 patients were evaluable at six months. Two of these 11 patients experienced a mild cutaneous reaction not necessitating D-pen withdrawal. Thus, nine (47%) of the 19 patients included initially experienced side effects, leading to stopping the D-pen treatment in seven cases (41%). Neither haematological side effects nor significant proteinuria were seen.
At baseline, seven out of the 15 subjects had positive IgA-Rf levels (>5 IU/ml) while 11 had positive IgM-Rf levels (>10 IU/ml). In *table 2* data are given on clinical parameters prior to and after D-pen treatment. There was a significant increase in basal salivary flow after three months. Although values of both basal salivary and stimulated parotid salivary flow at three and six months were higher compared with baseline values, the differences were not statistically significant. There were no subjective improvements as recorded by VAS. With respect to laboratory parameters (*table 3*) we found significant reductions of ESR and levels of IgM-Rf and IgA-Rf. Reductions in levels of IgM-Rf and IgA-Rf were impressive. Levels of IgG also decreased but this was not statistically significant.

DISCUSSION

Up to now results of treatment of primary Sjögren's syndrome (PSS) with respect to the function of lacrimal and salivary glands are very disappointing. Experience with D-pen treatment in PSS is scarce though, theoretically, this agent might be effective. We undertook the first prospective protocolised study on D-pen treatment in PSS. This open, uncontrolled study was intended to be a pilot study. There were relatively many dropouts, only 11 (58%) out of the 19 patients initially included completed the study. Side effects were frequently observed, especially severe (reversible) loss of taste. Possibly, PSS patients are more sensitive to loss of taste because of their lack of saliva. Side effects in PSS patients probably occur much more frequently compared with rheumatoid arthritis.
We found significant serological effects following D-pen treatment, including significant reductions in ESR and levels of IgA-Rf and IgM-Rf. Especially impressive were the reductions in the levels of IgM-Rf and IgA-Rf. However, clinical effects after D-pen were limited to only a statistically significant increase in basal salivary flow after three months. One could speculate whether the strong reduction in levels of IgM-Rf and IgA-Rf are causally related to the increase in basal salivary flow.

Table 2

Clinical parameters (mean ± SD) prior to and after three and six months of treatment with D-penicillamine in patients with primary Sjögren's syndrome

	D-PENICILLAMINE TREATMENT		
	PRIOR TO (N=15)	AFTER THREE MONTHS (N=15)	AFTER SIX MONTH (N=11)
Schirmer test (mm/5 min)	4.4 (± 4.4)	6.1 (± 1.6)	5.4 (± 1.9)
Tear break-up time (seconds)	3.6 (± 2.1)	4.4 (± 3.6)	3.7 (± 1.9)
Salivary flow			
- Baseline (ml/15 min)	1.8 (± 2.2)	2.7 (± 3.2)*	3.0 (± 3.8)
- After stimulation (ml/10 min)	4.0 (± 2.7)	4.1 (± 3.3)	5.2 (± 3.6)
Dry eyes#	5.0 (± 1.4)	4.0 (± 2.7)	2.5 (± 3.3)
Sand in the eyes feelings#	5.0 (± 1.4)	4.0 (± 2.7)	2.5 (± 3.4)
Dry mouth#	5.8 (± 1.6)	6.3 (± 2.0)	2.5 (± 3.3)

* *P=0.042 versus prior to treatment, # VAS (visual analogue scale) = severity of symptoms as scored on a scale from 0 (no symptoms) to 10 (most severe symptoms).*

Table 3
Laboratory parameters (mean ± SD) prior to and after three and six months treatment with D-penicillamine in patients with primary Sjögren's syndrome

	D-PENICILLAMINE TREATMENT		
	PRIOR TO (N=15)	AFTER THREE MONTHS (N=15)	AFTER SIX MONTHS (N=11)
ESR (mm/first hour)	23.7 ± 22.6	23.7 ± 20.8	21.5 ± 23.6*
Haemoglobin (mmol/l)	8.5 ± 0.8	8.6 ± 0.9	8.7 ± 1.0*
IgA (g/l)	2.5 ± 1.1	2.4 ± 1.0	2.2 ± 0.8#
IgG (g/l)	13.2 ± 3.9	13.3 ± 4.4	11.8 ± 3.3
IgM (g/l)	1.6 ± 1.8	1.3 ± 1.3*	0.8 ± 0.6#
IgA-Rf (IU/ml)	37.5 ± 64.7	25.7 ± 50.0*	10.5 ± 19.6
IgM-Rf (IU/ml)	27.4 ± 61.0	22.5 ± 55.4*	5.5 ± 7.4
β_2-microglobulin (mg/l)	2.5 ± 1.1	2.6 ± 1.1	3.0 ± 1.6

*ESR = erythrocyte sedimentation rate, Ig = immunoglobulin, Rf = rheumatoid factor, * p<0.05 versus prior to, # p<0.02 versus prior to.*

Indeed, it has been reported that levels of IgA-Rf are inversely related to stimulated parotid salivary flow.[15] The feeling of sand in the eyes and dry mouth as measured by VAS after six months seemed to improve although not statistically significantly.

The relationship between autoantibodies (including rheumatoid factors) observed in PSS and its disease manifestations such as xerostomia and xerophtalmia is far from clear. Rheumatoid factors and other immunoglobulins are produced locally in salivary glands in PSS.[13,14] In the past it has been reported that the synthesis of immunoglobulins in the salivary glands does not correlate with serum immunoglobulin levels.[19] Perhaps this is, partly, the explanation for the discrepancy between the relatively small clinical benefit and the strong serological effects of D-pen in PSS.

Our patients had established PSS for a mean of 3.8 years implicating (partly) irreversible destruction of gland tissue. Possibly, a stronger clinical response might be observed when PSS is treated at an earlier stage of the disease when there is more active inflammation.

Our results are largely comparable with the findings of Kruize *et al.*, who could not demonstrate any beneficial clinical effect after treatment of PSS with hydroxychloroquine.[1] They found a reduction in serum levels of IgG and IgM but not of the ESR, IgA and rheumatoid factors. Because of the high rate of side effects and the small clinical benefit, we cannot recommend D-pen as systemic treatment for patients with long-lasting PSS. However, it might be that D-pen is more beneficial in PSS patients with earlier disease. A prospective, double-blind, placebo-controlled trial might prove the potential role of D-pen in the treatment of early PSS.

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Renal graft failure due to type 1 primary hyperoxaluria

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ABSTRACT

Primary hyperoxaluria type 1 (PH1) usually presents with recurrent urolithiasis, nephrocalcinosis and progressive renal failure at a relatively young age. This report describes a patient who, due to the late onset of end-stage renal disease, had been diagnosed with PH1 only after failure of his second kidney graft. Retrospectively, his vascular problems, skeletal abnormalities and cardiac arrhythmias fit the picture of severe systemic oxalosis. Possible therapeutic options are discussed.

INTRODUCTION

Primary hyperoxaluria is a rare autosomal recessive disorder characterised by overproduction and excessive urinary excretion of oxalate. This results in recurrent urolithiasis, nephrocalcinosis, and systemic precipitation of calcium oxalate (oxalosis).[1-3] In primary hyperoxaluria type 1 (PH1), a functional defect of the hepatic peroxisomal alanine-glyoxylate aminotransferase (AGT) results in metabolic overproduction of oxalate and glycolate (*figure 1*).[2] In the vast majority of cases, PH1 presents in early childhood, the median age of onset being five to nine years.[4,5] Half of all the patients reach end-stage renal disease (ESRD) before the age of 15.[1,4] In the exceptionally rare and usually less severe type 2 primary hyperoxaluria (PH2), deficiency of D-glycerate dehydrogenase/glyoxylate reductase causes overproduction of oxalate and L-glycerate (*figure 1*).[6,7]
We present a patient with PH1 and an unusually late onset of ESRD, in whom the diagnosis was not made until after failure of his second renal graft. This case history clearly illustrates the clinical implications of systemic oxalosis. Moreover, the therapeutic options for this disease are discussed.

CASE REPORT

A 51-year-old Yugoslav man, whose medical history revealed an operation for urolithiasis at the age of three, developed ESRD at the age of 40 (1989), which was attributed to chronic obstructive nephropathy. Intermittent haemodialysis was started in Yugoslavia. In 1992 he left for the Netherlands for political reasons. In 1993 thrombosis of his arteriovenous fistula occurred, and a Goretex loop was constructed as an alternative access for haemodialysis. Hand X-rays showed bone resorptions and soft-tissue calcifications and radiographs of his pelvis showed osteosclerosis. An abdominal CT scan revealed small, massively calcified kidneys as well as calcifications in the pancreas and gallbladder. In subsequent years paroxysmal atrial fibrillation occurred for which pharmacological treatment was started. In 1995 left ventricular hypertrophy and mitral regurgitation was diagnosed. He was referred to the University Medical Centre St Radboud, Nijmegen, for a cadaveric kidney transplantation, which was performed in 1996. Severe acute interstitial and vascular rejection with subsequent thrombocytopenia necessitated removal of the graft, three weeks after transplantation. Despite oral anticoagulant treatment, thrombosis in his arteriovenous fistula occurred three times in the subsequent year. In 1997 he suffered from severe brady-tachy arrhythmias and he had to be resuscitated. Antiarrhythmic therapy was started and subsequently a pacemaker was implanted. In 2000, he complained of progressive claudication.

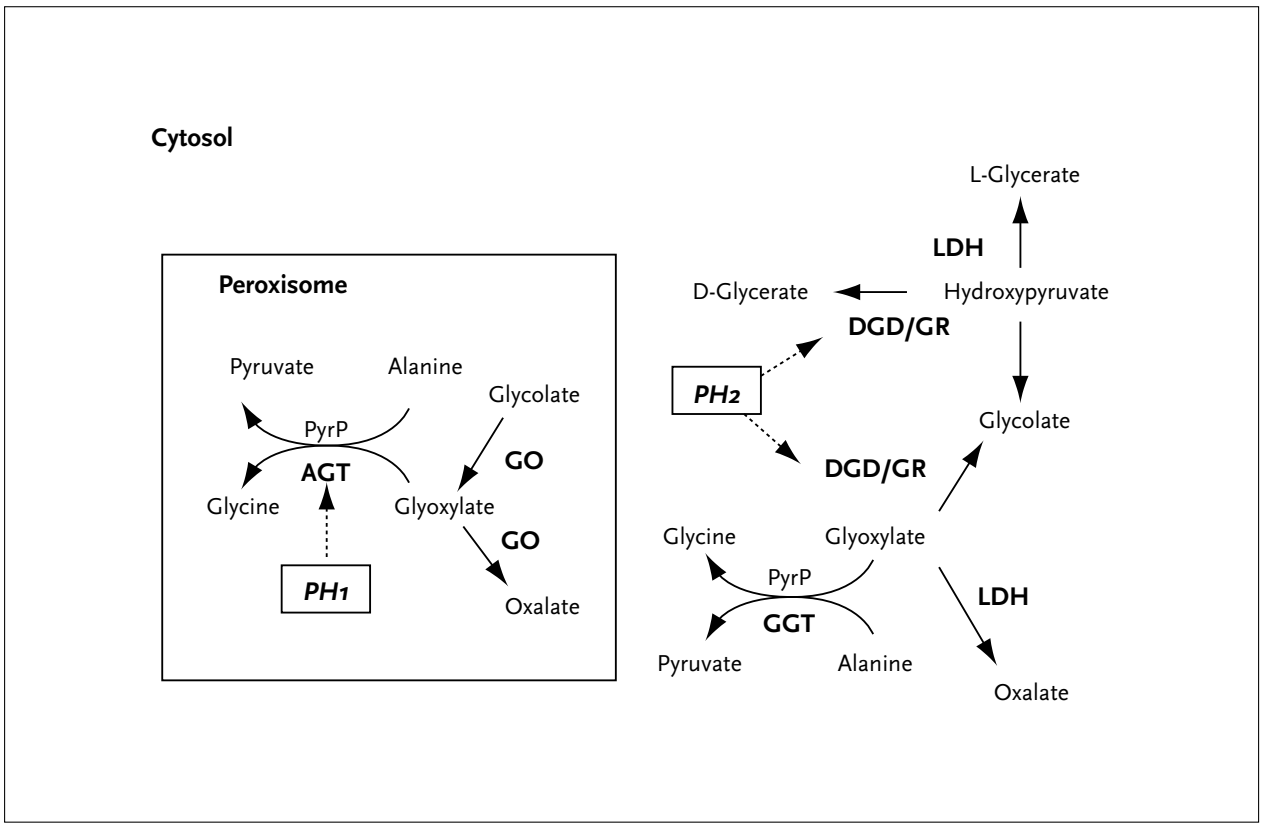

Figure 1
Schematic representation of some major pathways involved in glyoxylate, oxalate and glycolate metabolism in the human hepatocyte

AGT = alanine-glyoxylate aminotransferase, PyrP = pyridoxal phosphate, GGT = glutamate-glyoxylate aminotransferase, LDH = lactate dehydrogenase, DGD/GR = D-glycerate dehydrogenase/glyoxylate reductase, GO = glycolate oxidase.

Digital subtraction angiography of the lower extremities showed multiple arterial stenoses in the distal arteries. In September 2000 he underwent his second cadaveric kidney transplantation. As immunosuppressive therapy, he received a combination of prednisone, tacrolimus and mycophenolate mophetil. Creatinine clearance stabilised at a maximum of 28 ml/min three weeks after transplantation. On day 64 he was readmitted to the hospital because of a rise in the creatinine concentration from 220 to 310 μmol/l. Ultrasonography of the graft showed no abnormalities and rejection was suspected. Intravenous treatment with high doses of methylprednisolone was started, after a biopsy of the kidney graft was taken. The biopsy revealed numerous birefringent crystalline deposits in the proximal and distal tubules, arranged in a rosette-like array, consistent with calcium oxalate crystals, together with acute tubular necrosis. No signs of rejection were seen (*figure 2*). In the absence of any likely causes of secondary hyperoxaluria, primary hyperoxaluria was suspected. Biochemical urine analysis revealed elevated excretion rates of oxalate (284 μmol/mmol creatinine; reference range 0-80) and glycolate (197 μmol/mmol creatinine; reference range 0-120), whereas no L-glycerate was present. Plasma oxalate concentration was severely elevated up to 72 μmol/l (reference range <5 μmol/l). These investigations confirmed the diagnosis PH1. He was treated conservatively with a high fluid intake, avoidance of high oxalate foods, a thiazide diuretic, and a trial of pyridoxine. Eight months after his renal transplantation, the creatinine clearance had stabilised at 21 ml/min (plasma creatinine concentration 240 μmol/l) and the plasma oxalate concentration had decreased to 29 μmol/l. Repeated measurements of oxalate and glycolate will be performed to evaluate the further response to this conservative treatment regimen.[8]

DISCUSSION

Manifestations of systemic oxalosis due to PH1 include urolithiasis and nephrocalcinosis (kidney), bone pain and

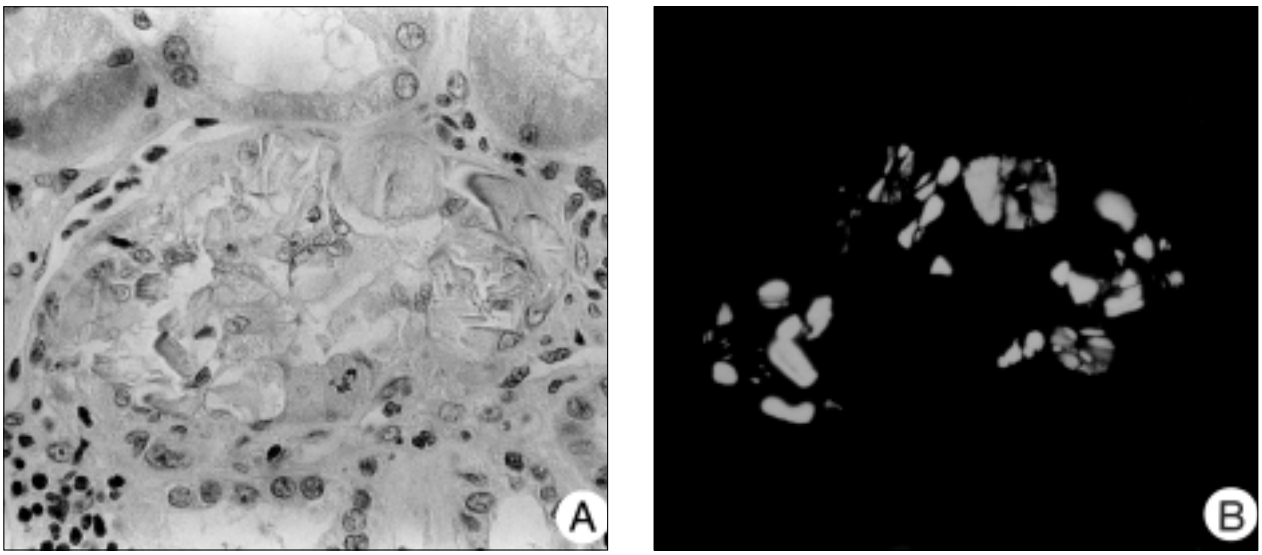

Figure 2
Light microscopic picture of a cross section of a tubulus with crystalline material accumulating in the lumen (A), which demonstrates birefringence with polarised light (B)

The composition and the colour (not seen in this black and white picture) are characteristic for oxalate (PAS stained section, x 450).

osteosclerosis (skeleton), retinopathy (eye), cardiomyopathy and arrhythmias including heart block (heart), disseminated occlusive vascular lesions and arteriovenous fistula thrombosis (arteries), peripheral neuropathy (nerves), synovitis (joints), and subcutaneous calcinosis and livedo reticularis (skin).[1,2,9] Median age of onset of ESRD is 15 to 25 years.[10] Considering these divergent symptoms as well as the considerable variation in the age of onset of ESRD, PH1 is a rather heterogeneous disorder.[11] Erroneously, because of recurrent urolithiasis, extensive calcifications of his native kidneys and especially the relatively late development of ESRD, a diagnosis of distal renal tubular acidosis was initially made in our patient. In recurrent nephrolithiasis with main constituents of stones other than calcium, a specific diagnosis as cysteinuria or even rarer types like (dihydroxy)adenine stones can generally be made by crystal or chemical stone analysis. In recurrent stones with calcium oxalate, phosphate or struvite as one of the main constituents, conditions such as hyperparathyroidism, medullary sponge kidneys, distal tubular acidosis, enteric hyperoxaluria and recurrent urinary tract infections with or without anatomic abnormalities should all be considered in the differential diagnosis.[12,13]
Moreover, attention should be paid to special risk factors for stone disease (high urinary concentration of stone constituents or low levels of crystal inhibitors, family history, diet, etc.). In our patient, the diagnosis of PH1 was not made until after failure of his second kidney graft. However, in retrospect, the clinical picture of our patient fits completely with a diagnosis of systemic oxalosis. Because PH1 is a rare and clinically very heterogeneous disease, the diagnosis is usually delayed by more than five years, except among infants.[10] The diagnosis of PH1 can be made by measuring the urine oxalate and glycolate excretion rates and by plasma oxalate measurement. Concomitant hyperoxaluria and hyperglycolic aciduria are indicative of PH1.[1] The presence of hyperglycolic aciduria is the most important parameter to distinguish PH1 from secondary hyperoxaluria and other forms of hyperoxaluria.[2]
However, some patients with PH1 have isolated hyperoxaluria without hyperglycolic aciduria.[2] Moreover, sporadic cases have been described of concomitant hyperoxaluria and hyperglycoluria in patients with normal AGT activity.[14] Therefore, for a definitive diagnosis of PH1, assessments of AGT activity and immunoreactivity in hepatic tissue are required.[10] This is particularly important if liver transplantation is considered as a therapeutic option. In our patient, a liver biopsy to confirm AGT deficiency was considered of no additional value because of the lack of therapeutic consequences.
Before renal failure occurs supportive measures are important to limit the concentrations of oxalate and calcium in the urine, preferably below 0.4 and 4 mmol/l respectively,[15] to decrease the risk of stone formation and progressive nephrocalcinosis. This can be achieved by a high fluid intake (>2 l/m^2/24 h), supplements of calcium oxalate crystallisation inhibitors (orthophosphate and sodium citrate or potassium citrate), if renal function allows the use of

these substances. Concomitant use of thiazide diuretics can decrease calcium excretion.[3,15] Avoidance of high oxalate foods, such as tea, chocolate, spinach and rhubarb, has only limited effects.[15] Responsiveness to 2 to 15 mg/kg/day of pyridoxine, a cofactor of the AGT enzyme pathway, has been reported in 10 to 40% of patients.[15] Combined treatment with orthophosphate and pyridoxine was reported to preserve renal function over a ten-year follow-up period.[16] After kidney transplantation, as excessive production of oxalate in the liver continues unabated, these conservative measures are important to maximise graft survival.[1]

Once PH1 has progressed to ESRD, conventional haemodialysis regimens are insufficient to prevent further systemic oxalate accumulation,[4] making early transplantation the treatment of choice in patients with progressive renal failure. Transplantation should preferably be performed before the glomerular filtration rate drops below 20 to 30 ml/min, because oxalate retention and subsequent systemic oxalosis then increases rapidly with a consequent decrease of graft survival rate.[4,17] In addition to early transplantation, vigorous haemodialysis immediately before and after this procedure is said to improve graft survival.[18] There is still no consensus about isolated kidney transplantation (KTX) or combined kidney-liver transplantation (K/LTX) being the strategy of choice. In Europe, the approach is directed to K/LTX more than to KTX, mainly because earlier studies on KTX showed a three-year graft survival of only 17 to 23%,[19] whereas, a more recent study on K/LTX showed one-, two-, and five-year patient survival rates of 88, 80, and 72% and graft survival rates of 82, 78, and 62%, respectively.[20] In the United States, however, a recent study concluded that KTX offered better patient survival than K/LTX (six-year survival 84 and 56% respectively).[21] Because of these inconsistencies and difficulties comparing the studies, an individual strategy is required.[1] Theoretically, K/LTX would be preferable in our patient, when renal function further deteriorates. His age and poor cardiovascular condition, however, make this a hazardous operation. If there is a further decline in renal function, a third KTX, with intensive preoperative and postoperative haemodialysis and continuation of supportive measures seems to be a better option.

In conclusion, considering the heterogeneity of PH1, this diagnosis should always be kept in mind in cases of unexplained renal failure, even in middle aged and older patients, if signs of systemic oxalosis are present.

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Netherlands
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Reversible migratory osteoporosis in renal oncocytoma mimicking renal cell carcinoma with bone metastases

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ABSTRACT

We report a case in which initially the wrong diagnosis of renal cell carcinoma with bone metastases was made. Nephrectomy and bone biopsy led to the right diagnosis of oncocytoma with transient osteoporosis. This report stresses the importance of pathological investigation and points to oncocytoma in the differential diagnosis of solid renal masses. In addition, the possible relationship between this tumour and migratory osteoporosis, which disappeared after surgery, is described.

INTRODUCTION

Oncocytes are transformed epithelial cells rich in mitochondria, probably representing senescent degenerative cellular changes. Renal oncocytoma is a benign tumour of renal tubular origin. Its incidence represents 3 to 10% of all solid renal masses.[1] Most renal oncocytomas are found incidentally and usually follow a benign clinical course. Partial nephrectomy or enucleation has been advocated as curative.[2] Here, we present a case of renal oncocytoma associated with bone lesions, which were due to migratory osteoporosis and muscle weakness.

CASE REPORT

A 42-year-old man presented with muscle weakness. For three years, he had been suffering from pain in his arms and legs not associated with trauma. He complained of progressive weakness of his shoulder and upper leg muscles and could not walk alone without help. He was not taking any medication. On physical examination, we saw a patient with atrophic shoulder and upper leg muscles and in the right part of the abdomen a large tumour was palpable. His weight was stable at 68 kg with a length of 1.70 m. Further examination showed no abnormalities. Laboratory examination revealed normal calcium (2.3 mmol/l), normal phosphate (0.8 mmol/l), elevated alkaline phosphatase 132 U/l, normal liver enzymes, and normal creatinine. Haematology showed no abnormalities; the ESR was 14 mm/h. Urine analysis revealed no erythrocyturia or leucocyturia. Electromyography was compatible with myopathy. Computer tomography of the abdomen showed a renal tumour (22 x 13 cm) in the right kidney without invasion in the adjacent structures (*figure 1*). There was

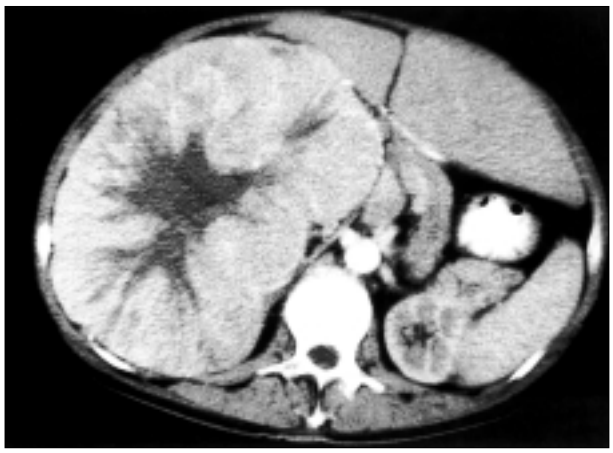

Figure 1
Computertomography of the abdomen, showing a renal tumour in the right kidney without invasion in the adjacent structures

either a central scar or necrosis inside the tumour. The X-ray of the thorax was normal. Nuclear bone scintigraphy revealed multiple abnormalities, suggesting metastases (*figure 2*). The diagnosis of renal cell carcinoma with bone metastasis was made. It was decided to perform a nephrectomy; on operation a giant tumour of the right kidney was found (*figure 3*). Microscopically the diagnosis of oncocytoma was made (*figure 4*). Histological examination of the biopsy taken of the humerus and sacrum at the site of abnormal uptake on the nuclear bone scintigraphy was compatible with migratory osteoporosis without metastases of the oncocytoma (*figure 5*). Two months after the operation the patient was able to walk again without help and the muscle weakness had disappeared. Six months after nephrectomy control scintigraphy of the bones showed disappearance of the initial bone lesions.

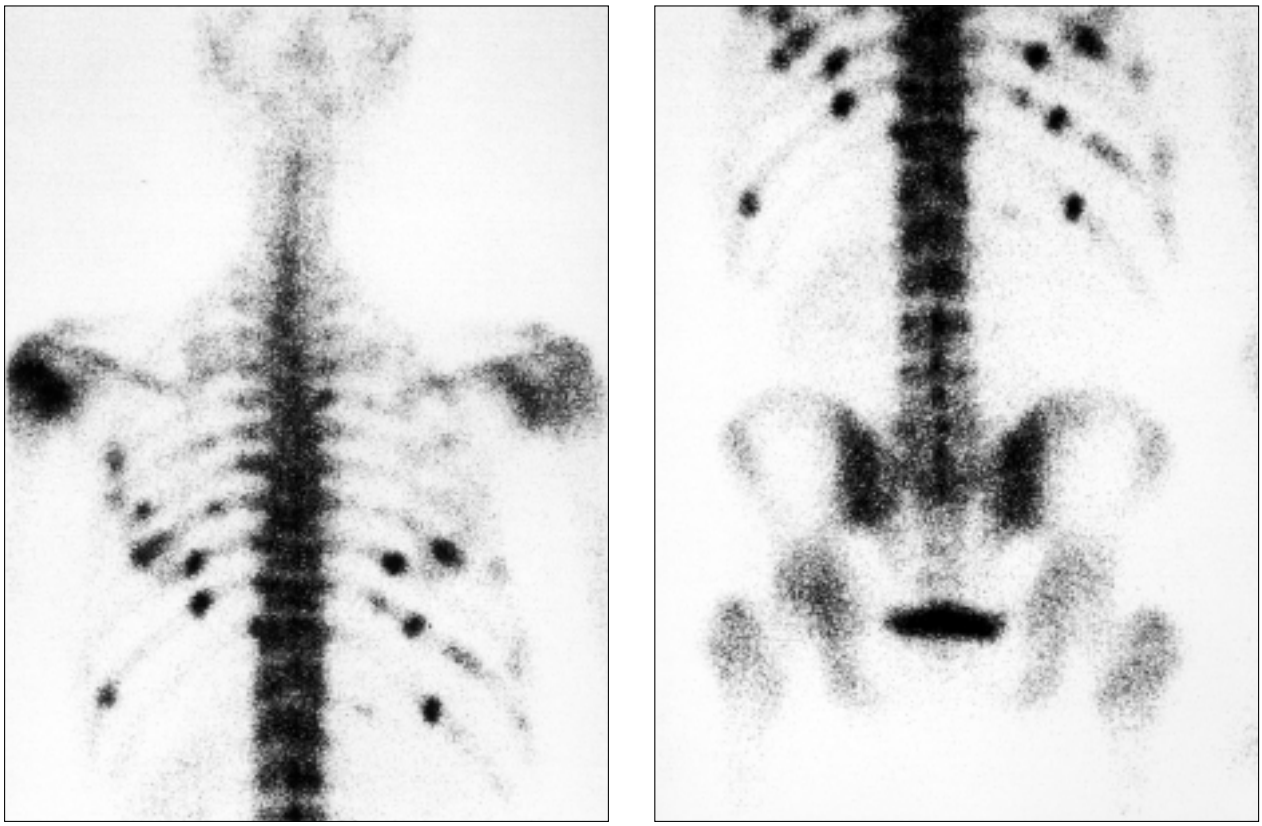

Figure 2
Nuclear bone scintigraphy, showing multiple abnormalities suggesting metastases

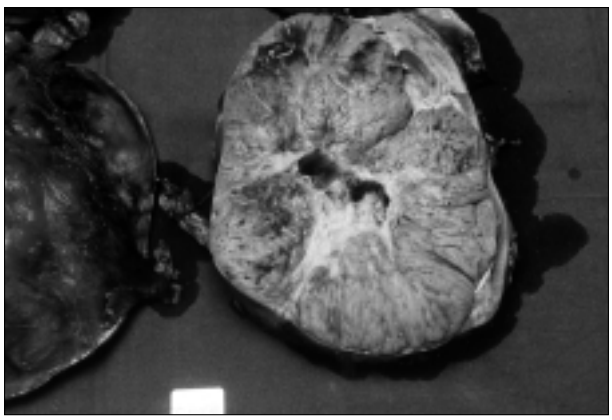

Figure 3
Macroscopic view of the right kidney; yellow/brown tumour with central scar, totally resected

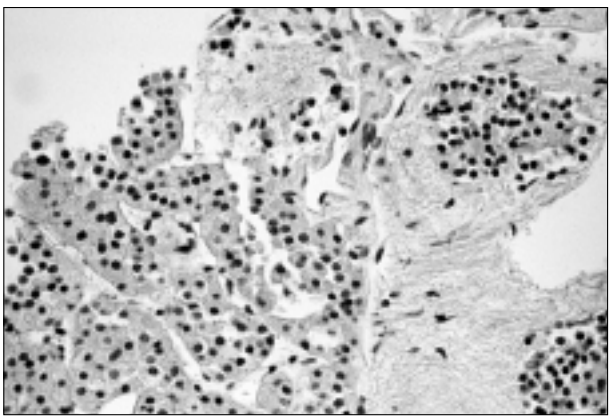

Figure 4
Core biopsy of the right kidney showing part of the central scar with nests of oncocytes

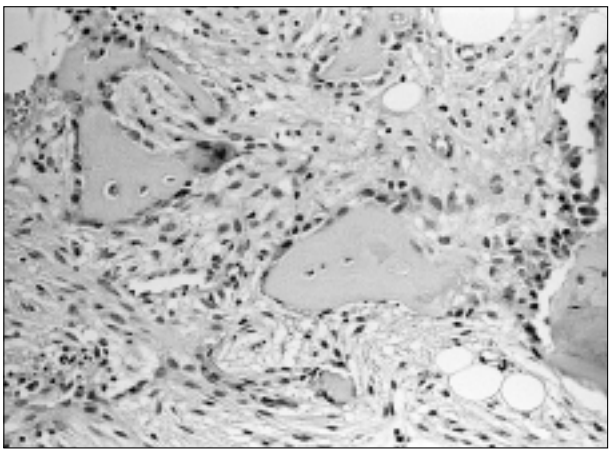

Figure 5
Core biopsy of the head of the humerus compatible with lesion on bone scintigraphy showing widening osteoid border, enhanced osteoclast activity and reactive bone formation suggesting migratory osteoporosis

DISCUSSION

In our patient we found an extremely large renal tumour and on nuclear scintigraphy a picture of bone metastases. Furthermore, there was a three-year history of generalised pain, predominantly in the upper and lower extremities and not associated with trauma. Initially, renal cell carcinoma with bone metastases was diagnosed. Nephrectomy led to the diagnosis of oncocytomas, which are usually discovered incidentally.[3] Sometimes, in a minority of cases, bilateral multifocal renal oncocytoma is found.[4] Because of the benign nature, multicentricity, possible bilaterality and absence of pathognomonic radiographic features, renal oncocytomas should considered in the differential diagnosis of solid renal masses. The fibrotic central scar in a larger mass, as in our case, has been described in oncocytoma and may be the most specific feature (*figure 3*).[5]
Fine needle biopsy should be considered to avoid radical nephrectomy in selected patients.
In this patient, biopsies of the decalcified ossal lesions of the bone scintigraphy showed characteristics of migratory osteoporosis: oedema, active osteoclastic bone resorption and reactive bone formation in marrow spaces (*figure 5*).[6]
Directly after nephrectomy all symptoms disappeared and a control bone scintigraphy six months after nephrectomy showed regression of the initial lesions. Probably the pain of the migratory osteoporosis and myopathy had induced the immobility leading to atrophic muscles.

We conclude that it is very likely that the ossal lesions had a pathogenetic relationship with this large oncocytoma in our patient.

Migratory osteoporosis is one of the two subgroups of transient osteoporosis.[7] The other is regional osteoporosis. Transient osteoporosis is a condition of pain mostly occurring in the lower limbs but other locations are described. It consists of monoarticular or oligoarticular pain in young and middle-aged persons associated with temporary rapidly progressing osteopenia. Pain increases with weight-bearing and can last for several months up to years. A significant proportion of patients have recurrences, sometimes in the same joint but mostly at shifting locations in the same or opposite limb. Intervals up to 13 years have been reported.
The aetiology and pathogenesis are poorly understood. Thrombotic aetiology has been suggested based on an abnormality of fibrinolysis seen in patients with transient osteoporosis. Transient osteoporosis has also been described as a variant of reflex sympathetic dystrophy with myopathy and muscle atrophy.[8] The treatment of transient osteoporosis is controversial; most cases are treated conservatively applying joint protection and cyclo-oxygenase inhibitors.
Furthermore, this case showed that an oncocytoma with paraneoplastic migratory osteoporosis can mimic a renal cell carcinoma with ossal metastases.

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Netherlands
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CASE REPORT

A young woman with fever and a pericardial effusion

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ABSTRACT

A 19-year-old woman is presented with high-spiking fever, pericardial tamponade and respiratory failure. A diagnosis of adult onset Still's disease was made. This is a rare inflammatory disease with an unknown aetiology. The diagnosis is made by exclusion and with the help of diagnostic criteria. Treatment with corticosteroids met with a good response.

INTRODUCTION

Fever is one of the most frequent presenting symptoms in medicine. Common causes of fever are infections, neoplasms or drug fever. Non-infectious inflammatory diseases can also cause fever. One of these is adult onset Still's disease. Although rare, this disease is among the most frequent causes of fever of unknown origin within the group of non-infectious inflammatory diseases.[1] In this case report we present a young woman with an unusual presentation of this disease.

CASE REPORT

A 19-year-old woman was admitted because of high-spiking fever of two weeks duration, accompanied by a dry cough, shortness of breath and painful muscles and joints. For some weeks, she had complained of a sore throat.
On examination we saw an ill, tachypnoeic patient with a temperature of 41°C, pulse 120 beats/min, blood pressure 100/60 mmHg. There was no rash. Two enlarged lymph nodes were palpated in her neck. Examination of the lungs and heart was within normal limits. Liver and spleen were moderately enlarged. Inspection of the joints showed no abnormalities.
The erythrocyte sedimentation rate was 35 (<12 mm/h), cAMP receptor protein 174 (<10 mg/l), haemoglobin 6.6 (7.2-9.8 mmol/l), mean corpuscular volume 84 (81-96 fl), leucocytes 4.8 (4.0-11.0 x 10^9/L) (peripheral smear normal), thrombocytes 127 (150-400 x 10^9/L). Slightly elevated values were obtained for the aspartate aminotransferase (ASAT) at 48 (<45 U/l), alanine aminotransferase (ALAT) at 75 (<45 U/l) and lactate dehydrogenase (LDH) at 668 (<475 U/l). Arterial blood gas (without supplemental oxygen): pO_2 7.7 (10-13 kPa), pCO_2 4.4 (4.5-6.0 kPa). The chest X-ray showed an enlarged cardiac silhouette and a small infiltrate. The electrocardiogram was normal. Our first suspected diagnosis was an infectious disease, although other diagnoses such as non-infectious inflammatory diseases or (haematological) neoplasms were considered. The patient was treated for a suspected pneumonia with cefuroxim and erythromycin. Cultures of blood and urine were negative. Extensive serology in paired sera for infections caused by *Mycoplasma, pneumonine Chlamydia spp., Legionella spp.* and adenovirus was negative.
Autoimmune tests were negative (antinuclear antibody (ANA), antineutrophil cytoplasmic antigen and rheumatoid factor). Ferritin was 347 (14-150 µg/l). Ultrasound revealed hepatosplenomegaly and a pericardial effusion. Her clinical condition deteriorated, with high swinging fever (*figure 1*), respiratory failure with bilateral infiltrates and impending pericardial tamponade. She was intubated and received artificial ventilation; just prior to this she was

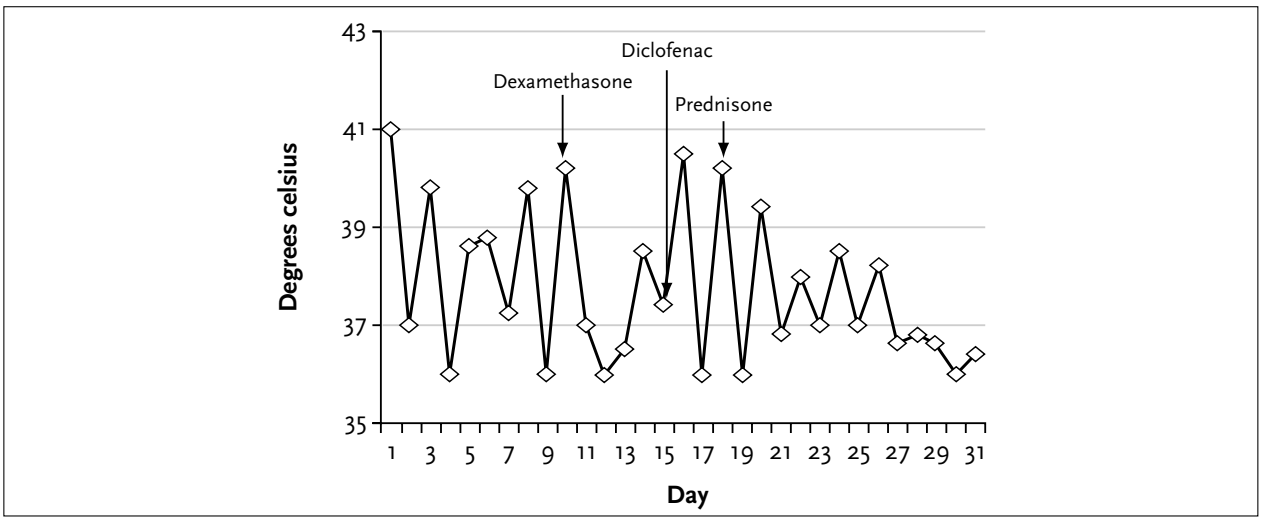

Figure 1
Temperature chart

given dexamethasone 100 mg iv. The pericardial effusion, which appeared to be a transudate, was drained (250 ml); cultures of this fluid were negative. Within 24 hours her condition improved dramatically and she was extubated. Because adult onset Still's disease was suspected, we stopped the antibiotics and started treatment with diclofenac: 3 x 50 mg orally per day on day 15. When the fever recurred and liver-enzyme abnormalities increased, prednisone was prescribed in an oral dose of 40 mg daily. Again the improvement was striking (*figure 1*). She was discharged in good condition, the laboratory abnormalities normalised. The steroids were tapered successfully over a few months.

DISCUSSION

Adult onset Still's disease is a rare form of seronegative polyarthritis of unknown aetiology. It is characterised by sudden onset of a high-spiking fever, a passing erythematous or salmon-coloured maculopapular rash involving the trunk and extremities (90%), polyarthritis or oligoarthritis (95%) and peripheral lymph node enlargement (50%). Splenomegaly, hepatomegaly, pericarditis and transient pulmonary infiltrates have been described in 30 to 40% of patients.[2] Cardiac tamponade and respiratory failure, as was the case in our patient, is a very rare complication.[3,4] Laboratory findings are aspecific with signs of inflammation and elevations of ASAT and ALAT, and LDH. Serum ferritin is markedly elevated in 70% of patients.[5] Characteristically all autoimmune tests are negative. The diagnosis is made by exclusion. Diagnostic criteria are divided into major criteria: fever of at least 39°C lasting for at least one week, arthralgias or arthritis lasting for two weeks, characteristic rash and leucocytosis; and minor criteria: sore throat, lymph node swelling, hepatomegaly or splenomegaly, abnormal liver enzyme tests, negative tests for ANA and rheumatoid factor.[6,7] To establish the diagnosis a patient should fulfil five of the criteria listed above, as did our patient (two major and all minor criteria). First-line therapy for adult Still's disease is NSAIDs (about 25% of patients react to NSAIDs). Alternatively corticosteroids (0.5-1 mg/kg/day) can be given. In patients with high fever of longer duration, pericardial effusion and pulmonary infiltrates, in the absence of infectious or autoimmune causes, adult type Still's disease should be suspected and a trial of corticosteroids is warranted.

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ANSWER TO PHOTO QUIZ (ON PAGE 389)
COLLAPSE AFTER MODERATE EXERCISE –
L.E. OOSTENBRUG, T.S. VAN DER WERF, W.F. HEESEN

The echocardiography showed a thickening of the interventricular septum that is diagnostic for hypertrophic cardiomyopathy (HCM). HCM is a relatively common genetic cardiac disease, 1:500 in the general population, with a variable genetic pattern and variable clinical expression. It is heterogeneous in presentation and prognosis.[1] Although it is the most common cause of sudden cardiac death in the young, it is an uncommon cause of sudden cardiac death in adults;[2,3] annual mortality is around 1%.[4] Myocyte disarray is associated with the occurrence of arrhythmias.[5] In our patient, this was also the predominant finding at postmortem examination.

If cardiopulmonary resuscitation is successful for syncopal sustained ventricular tachycardia/ventricular fibrillation, patients may benefit from an implantable cardioverter defibrillator[6] that offers better protection than low-dose amiodarone, but well-designed prospective trials comparing surgical,[7] ablative[8,9] and antiarrhythmic treatments[10] have not yet been conducted.

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ABOUT THE COVER

'Rivier' (River)

Ruud Matthes

This month's cover, entitled 'Rivier' (River), shows a print made by Ruud Matthes.

Ruud was born in Amsterdam, the Netherlands in 1948. While studying social pedagogy at the University of Amsterdam from 1972 to 1979, he also pursued studies in printmaking at the Free Academy in The Hague, the Netherlands (1975), at the International Summer Academy in Salzburg, Austria (1976) and at Atelier 17 of S.W. Hayter in Paris, France (1978). In 1989 he moved to Thessalonica in Greece. He usually presents his work in group exhibitions in Thessalonica and Athens. His last personal exhibition was in Gallery ZM in Thessalonica in 1999. For the last 20 years he has been inspired by the Greek landscape, which you will find in his prints.

A limited edition of original prints (size 29 x 21 cm) of this month's cover is available at a price of € 200. You can order the print at Galerie Unita, Rijksstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands or by e-mail: galerie-unita@planet.nl.

Aims and scope
The Netherlands Journal of Medicine publishes papers in all relevant fields of internal medicine. In addition to reports of original clinical and experimental studies, reviews on topics of interest or importance, case reports, book reviews and letters to the Editor are welcomed.

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Manuscripts submitted to the Journal should report original research not previously published or being considered for publication elsewhere. Submission of a manuscript to this Journal gives the publisher the right to publish the paper if it is accepted. Manuscripts may be edited to improve clarity and expression.

Language
The language of the Journal is English. English idiom and spelling is used in accordance with the Oxford dictionary. Thus: Centre and not Center, Tumour and not Tumor, Haematology and not Hematology.

Preparation of manuscripts
Type all pages with double spacing and wide margins on one side of the paper. To facilitate the reviewing process number the pages; also we would appreciate seeing the line numbers in the margin (Word: page set-up - margins - layout - line numbers). Divide the manuscript into the following sections: Title page, Abstract, Introduction, Materials and methods, Results, Discussion, Acknowledgements, References, Tables and Figures with Legends.

A *Covering letter* should accompany the manuscript, identifying the person (with the address, telephone and telex numbers, and e-mail address) responsible for negotiations concerning the manuscript: the letter should make it clear that the final manuscript has been seen and approved by all authors. Conflicts of interest, any commercial affiliations, consultations, stock or equity interests should be specified. In the letter 1-3 sentences should be dedicated to what this study adds. All authors should sign the letter.

The *Title page* should include authors' names, degrees, academic addresses, address for correspondence including telephone, fax and e-mail, and grant support. Also the contribution of each author should be specified.
The title should be informative and not exceed 90 characters, including spaces. Avoid use of extraneous words such as 'study', 'investigation' as well as priority claims (new, novel, first). Give a running title of less than 50 characters. If data from the manuscript have been presented at a meeting, list the name, date and location of the meeting and reference and previously published abstracts in the bibliography. Give a word count (including references, excluding tables and legends) at the bottom of this page.

Abbreviations: Measurements should be abbreviated according to SI units. All other abbreviations or acronyms should be defined on the first appearance in the text. Use a capital letter for proprietary names of substances and materials. At first mention of a chemical substance, use the correct chemical designation as well as the generic name.

The *Abstract,* not exceeding 200 words, should be written in a structured manner and with particular care, since this will be the only part of the article studied by some readers. In original articles, the abstract should consist of four paragraphs, labelled Background, Methods, Results, and Conclusions. They should briefly describe the problem being addressed in the study, how the study was performed and which measurements were carried out, the most relevant results, and what the authors conclude from the results.

The *Introduction* should be brief and set out the purposes for which the study has been performed.

The *Materials and methods* should be sufficiently detailed so that readers and reviewers can understand precisely what has been done without studying the references directly. The description may be abbreviated when well-accepted techniques are used.

The *Results* should be presented precisely without discussion.

The *Discussion* should directly relate to the study being reported. Do not include a general review of the topic, but discuss the pertinent literature.

Acknowledgement: All finding sources should be credited here. Also a statement of conflicts of interest should be put here.

References should be numbered consecutively (in square brackets) as they appear in the text. Type the reference list with double spacing on a separate sheet. References should accord with the system used in Uniform requirements for manuscripts submitted to biomedical journals (N Engl J Med 1991;324:424-8).

Examples:

[1.] Smilde TJ, Wissen S van, Wollersheim H, Kastelein JJP, Stalenhoef AFH. Genetic and metabolic factors predicting risk of cardiovascular disease in familial hypercholesterolemia. Neth J Med 2001;59:184-95.

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