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EDITORIAL

Pilot studies: one swallow does not make a summer ...

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ABSTRACT

What should we expect from pilot studies, done in small series of patients? In the literature there are many examples of small studies with very promising results, that in subsequent larger or better controlled studies proved to be much less promising, or even disastrous. In some instances the initial favourable outcome was due to selection bias. In others the use of nonvalidated methods of measuring outcome made the reproducibility of promising observations problematic. However, we have to start somewhere. In ths issue The *et al*. report favourable results of granisetron treatment in four out of five patients with chronic fatigue syndrome. A prospective, randomised, placebo-controlled, double-blind clinical trial with granisetron in patients with chronic fatigue syndrome is now ongoing.

In this issue of the Netherlands Journal of Medicine, The *et al*. report a pilot study of granisetron treatment in patients with the chronic fatigue syndrome.[1] They treated five patients, all meeting the CDC criteria for chronic fatigue syndrome, with a four-week course of granisetron in an uncontrolled study. Using validated assessment instruments measuring fatigue severity and functional impairment, they found improvements in four out of five patients. Although due to the design of the study a placebo effect can not be excluded, the authors state that they have not encountered similar remarkable changes in fatigue severity and functional impairment in the placebo groups of previously performed placebo-controlled studies. The results of this pilot study have made them initiate a prospective, randomised, placebo-controlled, double-blind clinical trial with granisetron in patients with the chronic fatigue syndrome.

What should we expect from drugs evaluated in studies such as the one by The and colleagues? Treatment of patients with the chronic fatigue syndrome is not an easy task. Prescribing a drug is a lot easier than interventions such as cognitive behaviour therapy or graded exercise therapy. Although we would all be very happy with an effective drug therapy for chronic fatigue syndrome, the size of this pilot study does not permit high expectations. In the literature there are too many examples of studies with very promising results, which in subsequent larger or better controlled studies proved to be much less promising or even disastrous.

A first example is the use of nitric oxide synthase (NOS) inhibitors in the treatment of septic shock. Vascular endothelial cells make nitric oxide. This endothelium-derived nitric oxide stimulates cyclic guanosine monophosphate synthesis in the underlying vascular smooth muscle, causing relaxation. Overproduction of nitric oxide can lead to inappropriate vasodilation, loss of systemic vascular resistance and hypotension. In septic shock, such an overproduction of nitric oxide is attributable to a distinct, high output, cytokine- and endotoxin-inducible NOS isoform (iNOS) present in endothelial and/or smooth muscle cells.[2] Following encouraging studies in experimental settings clinical trials investigating the effects of treatment with an NOS inhibitor, N^G-methyl-L-arginine (L-NMMA), were initiated. In 1999, Grover *et al*. published the results of a multicentre, dose-ranging, safety study of 546C88 (NMA hydrochloride) for the treatment of septic shock

(n=32). This compound proved to be a potent vasoactive agent capable of restoring systemic vascular resistance, reducing or eliminating the need for concurrent epinephrine therapy.[3] A subsequent placebo-controlled, multicentre phase III trial was terminated after inclusion of almost 800 patients, when a safety analysis found significantly worse survival (p<0.005) among patients receiving 546C88. The adverse outcome can possibly be explained by the fact that L-NMMA not only inhibits the inducible NOS, but also constitutive isoforms of NOS. The primarily cardiac serious adverse events may have been due to direct cardiac toxicity of L-NMMA.[4] Development of NOS isoform-selective inhibitors may produce agents with a larger therapeutic index. A promising new treatment modality came to an early end.

Another example of a study with disappointing results is the ELITE II study.[5] The ELITE II study was the successor of ELITE I.[6] In ELITE I elderly patients (n=722) with symptomatic heart failure (NYHA class II-IV) were treated (double-blind) with losartan titrated to 50 mg once daily, or to 50 mg of captopril three times daily, for 48 weeks. An unexpected 46% lowering of mortality (a secondary endpoint) was observed with losartan compared with captopril (losartan 17 (4.8%) *vs* captopril 32 (8.7) deaths; risk reduction 46% (95% CI: 5-69%); p=0.035). In addition, losartan reduced the rate of all-cause hospital admissions, and was better tolerated than captopril, despite a similar persistent rise in serum creatinine concentrations (primary endpoint of the study). The apparent superior effects seen with losartan on morbidity and mortality were based on a small number of events that were not the primary endpoint. Therefore, a much larger, randomised double-blind trial, ELITE II, was designed to compare the effects of losartan with those of captopril on mortality, morbidity, safety and tolerability. In ELITE II, 3152 patients aged 60 years or older with heart failure (NYHA class II-IV) and ejection fraction <40%, were treated (double-blind) with losartan titrated to 50 mg once daily, or to 50 mg of captopril three times daily. Disappointingly, in this study losartan did not prove to be superior to captopril in improving survival. Mortality and sudden death did not differ significantly between groups. ELITE II did confirm the superior tolerability of losartan observed in ELITE I.

A third example of gradually decreasing enthusiasm is the story of recombinant activated protein C (rhAPC). In March 2001 investigators reported the results of a phase III trial enrolling 1690 patients with severe sepsis showing that rhAPC significantly reduced the absolute risks of death from 30.8% in the placebo group to 24.7% in the treatment group.[7] The prevalence of bleeding as a serious adverse event during the 28-day follow-up period was greater with rhAPC than placebo (3.5% *vs* 2.0%), but this difference did not reach statistical significance (p=0.06). In light of the high mortality with sepsis and lack of alternative therapies, these encouraging results were welcomed by many healthcare professionals who expected rhAPC to quickly become available for clinical use. However, the Food and Drug Administration (FDA) asked for additional phase III testing before a final decision about its use clinically was taken. The FDA, because of concerns, restricted its use to those with a high risk of death.[8] Further analysis of the phase III trial results showed that rhAPC was substantially more beneficial in the second than first half of the trial. This change in effect was associated with an amendment modifying trial enrolment criteria, and a change in the manufacturing of rhAPC.[9] Additional safety concerns were the high prevalence of serious intracranial haemorrhages reported in a compassionate use protocol, and the seemingly smaller efficacy in a less ill subpopulation. A similar relationship between risk of death and effect of treatment was also found for other mediator-specific anti-inflammatory agents.[10] Studies in septic patients with mild disease are underway. So far, rhAPC use is limited to severe sepsis only.

Fortunately not all initial good experience leads to disappointment later on. There are also many examples of promising small or uncontrolled studies that did turn out to become accepted therapies, on the basis of subsequent controlled clinical trials. Especially in the treatment of the acquired immunodeficiency syndrome AIDS, the early studies were mostly uncontrolled. Multiple randomised trials have led to the availability of 15 registered antiretroviral drugs in the Netherlands, which are now often used in combination therapy.[11]

Methotrexate is currently the most frequent choice of disease-modifying antirheumatic therapy for rheumatoid arthritis.[12] In the 1980s methotrexate was mostly used in individuals who had severe rheumatoid arthritis. Patients with more severe disease have a higher risk of cardiovascular death. In studies without adjustment for this confounding factor methotrexate use is linked to poor outcome.[13] However, uncontrolled observational studies suggested effectiveness of methotrexate for rheumatoid arthritis.[14,15] In double-blind, randomised studies the improvement of methotrexate on mobility and systemic inflammation was confirmed.[16]

A third example of the development of a successful therapy is the use of infliximab for Crohn's disease. *In vitro* studies suggested that the production of tumour necrosis factor α (TNF-α) in the mucosa of patients with Crohn's disease is increased.[17] Similar findings were reported for the synovia of patients with rheumatoid arthritis. In patients with rheumatoid arthritis, treatment with antibodies against TNF-α were found to reduce signs and symptoms of this disease.[18] This stimulated the use of anti-TNF-α in patients with Crohn's disease. In that preliminary trial of only nine patients, a remission occurred after one infusion of the antibody in eight patients.[19] In a subsequent double-blind,

placebo-controlled trial in 108 patients with treatment-resistant Crohn's disease the efficacy of the antibody was confirmed.[20]

The above is to illustrate that one swallow does not make a summer. Initial promising data need confirmation in larger, well-designed clinical trials. Only then will we be able to fully benefit from adequately tested drugs. Especially in diseases with a highly variable clinical course, such as sepsis, small uncontrolled series may suffer from important selection bias. The examples of rhAPC and L-NMMA show how careful we should be with the interpretation of pilot studies in the treatment of sepsis. Another reason for wrongful optimism after pilot studies is the use of nonvalidated methods of measuring outcome. Objective and accepted measures of disease activity, such as disability index scores in rheumatoid arthritis, make the reproducibility of promising observations more likely. Nevertheless, we have to start somewhere. Hopefully the concept of granisetron therapy for chronic fatigue syndrome will prove to be highly effective and safe. The good news is that upregulated serotonin seems to play a pathophysiological role in the neurobiology of chronic fatigue syndrome. This means that granisetron, a serotonin antagonist, is a rational therapy. Also, the methods to assess the effect of interventions are well established. We look forward to the results of the prospective, randomised, placebo-controlled, double-blind clinical trial with granisetron in patients with the chronic fatigue syndrome.

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REVIEW

Endothelial activation, endothelial dysfunction and premature atherosclerosis in systemic autoimmune diseases

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ABSTRACT

Atherosclerosis may be considered an inflammatory disease characterised by the development of atherosclerotic plaques and ischaemic cardiovascular events. Increased prevalence of cardiovascular morbidity and mortality due to (premature) atherosclerosis has been observed in patients with autoimmune diseases like rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Wegener's granolumatosis. This increased prevalence cannot be explained by the presence of the traditional cardiovascular risk factors such as hypertension, hyperlipidaemia, diabetes mellitus and smoking. Therefore, other risk factors must be present in patients with systemic autoimmune disease. Although the mechanisms have not been fully unravelled, endothelial cell (EC) activation through autoantibodies seems to be one of the factors involved. EC activation results in EC dysfunction. It is supposed that chronic EC dysfunction, as present in patients with systemic autoimmune disorders, contributes to the development of premature atherosclerosis and results in an increased prevalence of cardiovascular disease.

INTRODUCTION

The endothelium is a physical barrier between the blood and the underlying tissues. Healthy endothelial cells (EC) are essential for the maintenance of vascular homeostasis since they synthesise and express different vasoactive substances and molecules. EC are responsible for the continuous adjustment of vascular tone, control of blood pressure, regulation of leucocyte traffic from blood to tissues and the maintenance of antithrombotic and anticoagulant balance in flowing blood. They are also involved in the control of growth, development and differentiation of the vessel wall, and solute flux and fluid permeability across the vessel wall. Furthermore, EC are involved in platelet adhesion and aggregation, and blood coagulation and fibrinolysis.[1]

The vascular tone is controlled by synthesis and secretion of two vasodilators, (prostacyclin (PGI_2) and nitric oxide (NO), endothelium-derived hyperpolarising factor, CO and the vasoconstrictors endothelin-1, thromboxane A2 and endoperoxidase. The careful balance between these mediators provides minute-by-minute control of tone and blood pressure. Leucocyte migration is controlled by expression of adhesive molecules capable of attracting and firmly attaching leucocytes after stimulation with thrombin, cytokines or endotoxins. The main transmembrane proteins involved in the capture and rolling of leucocytes are P-selectin, E-selectin, L-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM-1).

A network of events that involves interaction of thrombin with EC controls platelet function, coagulation and fibrinolysis and contributes to the maintenance of normal blood fluidity by anticoagulant and antiplatelet effects. This process includes synthesis and secretion of NO, PGI_2, platelet-activating factor (PAF) and von Willebrand factor (vWF), and an increase in solute permeability between EC. For control of fibrinolysis, EC secrete tissue plasminogen activator (tPA) and its inhibitor plasminogen activator inhibitor-1 (PAI-1).

Loss or dysregulation of these homeostatic mechanisms, due to activation of EC, characterises EC dysfunction and results in an inflammatory response leading to atherosclerosis.[2] The inflammatory response is reflected by elevated

levels of high-sensitivity C-reactive protein (hsCRP) and seems to be a promising marker of atherosclerotic activity.[3,4] Prospective studies in the general population have shown that levels of hsCRP, but also of other acute-phase reactants like interleukin-1, are related to the future risk of cardiovascular disease.[5-7]

ENDOTHELIAL CELL ACTIVATION

EC can be activated by stimulation with different agents such as interleukin-1, several autoantibodies and modified low-density lipoproteins (LDL).[8] EC activation is characterised by five main changes: loss of vascular integrity, increased expression and shedding of leucocyte adhesion molecules, change in phenotype from antithrombotic to thrombotic, production of several cytokines, and upregulation of HLA molecules. These changes allow EC to participate in the inflammatory response. The enhanced expression of adhesion molecules and chemoattractants provides an environment for leucocyte adhesion and migration of these cells into the vessel wall. Thrombosis can occur. Furthermore, activation of leucocytes leads to release of enzymes, cytokines, chemokines and growth factors resulting in a cascade of events accumulating in smooth muscle cell proliferation and formation of plaques.[2,9]
In systemic autoimmune diseases like systemic lupus erythematosus (SLE) as well as in vasculitides such as Wegener's granulomatosis, inflammation of many organ systems can occur. The presence of EC activation has been demonstrated indirectly by the elevation of circulating levels of soluble adhesion molecules, thrombomodulin and NO.[10-18] Furthermore, surface protein expression of VCAM-1, ICAM-1 and E-selectin on EC is increased.[19]
The nature of this EC activation is unknown. It has been suggested that next to the presence of the classical risk factors for atherosclerosis, disease specific factors are involved. In particular, autoantibodies directed to phospholipids, endothelial cells, double-stranded DNA (dsDNA) and ribonucleoproteins (nRNP) can induce EC activation *in vitro*.[20-24] Also antineutrophil cytoplasmic antibodies (ANCA) are implicated in EC activation, although presumably to a lesser extent.[25] Most relevant seems the presence of antibodies to oxidised LDL (oxLDL) and to HSP65. Antibodies to these antigens are increased in patients with accelerated atherosclerosis and those directed to HSP65 have been shown to be an independent risk factor for cardiovascular disease.[26,27] Increased levels of anti-oxLDL antibodies have been found in patients with systemic vasculitis.[28] Next to the presence of EC-activating antibodies, also disturbances in the lipid spectrum, including increased levels of lipoprotein (a), elevated levels of total cholesterol and triglycerides, and elevated plasma levels of circulating oxLDL have been found in patients with SLE.[29,30] Parts of the changes in the lipid spectrum are possibly explained by the presence of antibodies to lipoprotein lipase (LPL) as the presence of these antibodies is strongly correlated with total serum triglycerides, apolipoprotein B and apolipoprotein E concentrations.[31]

ENDOTHELIAL CELL DYSFUNCTION

The EC activation and the inflammatory vessel wall damage in systemic autoimmune diseases and systemic vasculitis may result in EC dysfunction. EC dysfunction can be measured by pulse-wave analysis (PWA) or flow-mediated vasodilation (FMD). PWA is a technique in which large and small artery compliance is estimated from analysis of the peripheral arterial waveform. The radial artery is measured with a tonometer. Increased vascular stiffness is a sign of EC dysfunction and correlates with invasive tests of arterial compliance.[32] Large and small artery compliance is calculated from the recorded waveform. Reduced arterial compliance has been shown to predict coronary events and mortality in patients with hypertension.[33] Recently, in a small group of 18 RA patients, free of traditional cardiovascular risk factors, PWA measurement showed a marked decrease of arterial compliance. In the same patient group, FMD was normal, suggesting that PWA is probably a more sensitive marker of vascular dysfunction in RA.[34,35]
FMD determines with ultrasound the capability of the brachial artery to dilate after the occlusion of the forearm by inflation of a pneumatic tourniquet. Deflation of the tourniquet increases blood flow to the distal part of the forearm, inducing an endothelium-dependent vasodilatation. The results obtained are then compared with results obtained after nitroglycerine (NTG) sublingually, resulting in endothelium-independent vasodilation. EC dysfunction is present when only the EC-dependent vasodilation is impaired. The technique has been validated and the results obtained have been shown to relate to the extent and severity of coronary artery disease.[36,37] In a small group of patients with primary systemic necrotising vasculitis (n=24) endothelial function was assessed by FMD as described above. Indeed, in these patients endothelium-dependent FMD was severely impaired compared with age- and sex-matched controls ($p<0.0001$).[38] Endothelium-dependent FMD was impaired in patients with active disease in particular and improved after suppression of the inflammation in all seven patients with active vasculitis who where measured before and after treatment ($p=0.016$). In SLE patients endothelium-dependent FMD was analysed cross-sectionally in 69 patients. Also in these patients endothelium-dependent FMD was significantly impaired compared with controls, even in the subgroup of patients without coronary artery disease risk factors ($p<0.001$).[39]

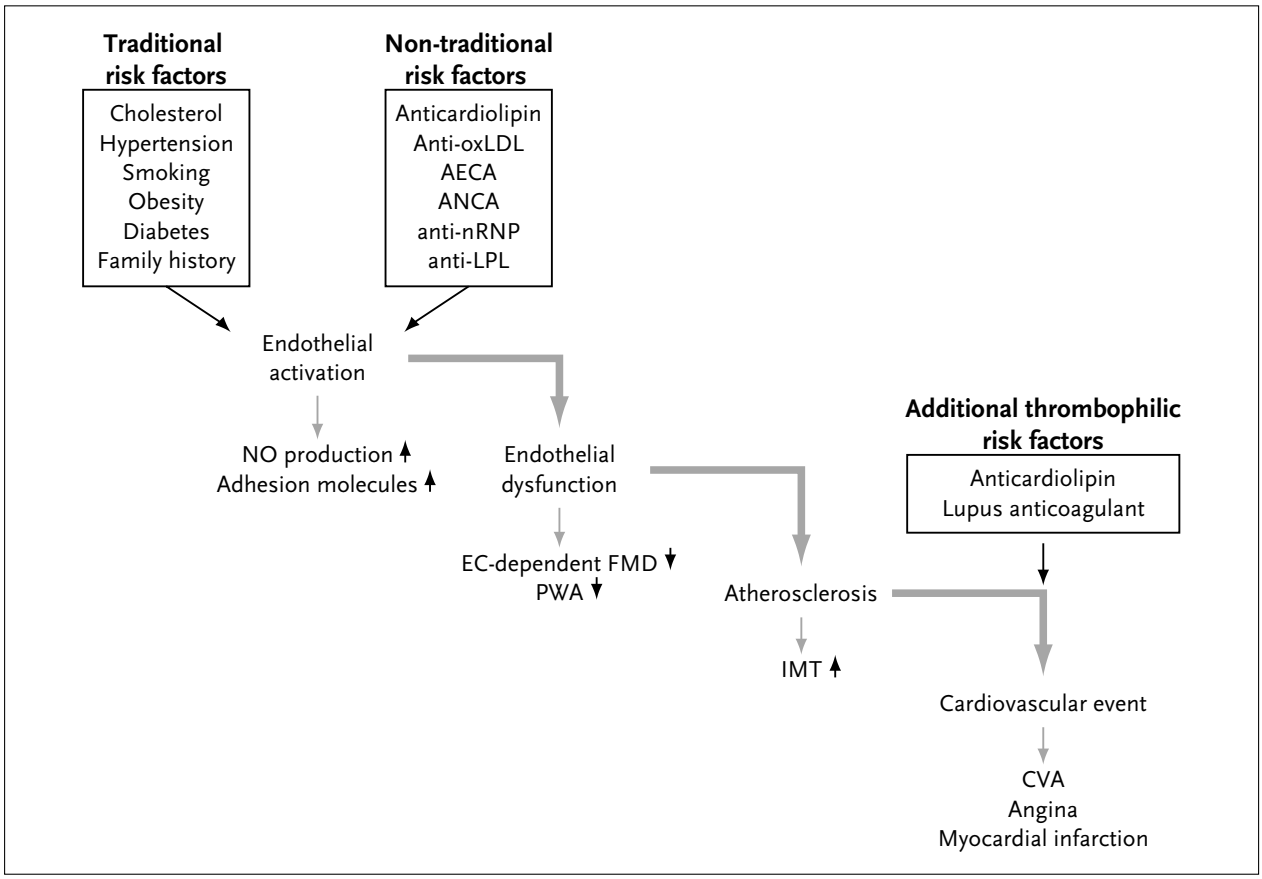

Figure 1

Simplified schematic view of the development of cardiovascular disease in patients with systemic autoimmune disease

Anti-ox LDL = antibodies to oxidised LDL; AECA = antiendothelial cell antibodies; ANCA = antineutrophil cytoplasmic antibodies; anti-nRNP = antibodies to nuclear ribonucleoprotein; anti-LPL = antibodies to lipoprotein lipase, NO = nitric oxide; FMD = flow-mediated vasodilation; PWA = pulse-wave analysis; IMT = intima-media thickness. Next to the traditional cardiovascular risk factors additional factors are involved in EC activation. EC activation results in EC dysfunction that can be measured using PWA or FMD. Severe, or chronically impaired EC dysfunction results in premature atherosclerosis. Atherosclerotic plaques can be detected by measuring IMT in the carotid artery. These lesions are thrombogenic and might therefore cause thrombosis or plaque rupture and overt cardiovascular disease. Also in the process of thrombosis additional risk factors play a role in patients with autoimmune disease. Especially the presence of thrombophilic factors like antiphospholipid antibodies increase the risk of cardiovascular disease.

Interestingly, the endothelium-dependent dilation was not related to disease duration, cumulative prednisone dose, use of antimalarial agents, anticardiolipin antibody, Raynaud's phenomenon or presence of vasculitis.

ATHEROSCLEROSIS

It can therefore be hypothesised that EC dysfunction, as present in patients with systemic autoimmune disorders, reflects EC activation and when severely and/or chronically impaired results in the development of premature atherosclerosis. Indeed, next to the studies showing impaired FMD in systemic vasculitis and in SLE, studies have been performed to evaluate whether premature atherosclerosis could be detected in these patients.

Studies of carotid and intima-media wall thickness (IMT) in unselected SLE patients showed that 40% of the patients had at least one focal plaque and a high risk for cardiovascular disease.[40] It is of interest to note that traditional risk factors seem to be insufficient to explain the increased prevalence of atherosclerosis and cardiovascular disease found in SLE patients.[41] The mortality due to myocardial infarction in SLE patients compared with age- and sex-matched controls are increased up to 50-fold.[42] In patients with rheumatoid arthritis the clinical findings are comparable, although less impressive. The risk of death from cardiovascular disease in RA patients is doubled.[43,44] Similarly to the findings in SLE patients, in rheumatoid arthritis patients without a history of atherosclerosis or its complications, IMT was significantly greater than in age- and sex-matched controls.[45]

INTERVENTION

HMG-coenzyme A-reductase inhibitors (statins) are potent lipid-lowering drugs. Next to their cholesterol-lowering capacity they have other effects. Statins are able to reduce the expression of adhesion molecules on monocytes and diminish their adhesion to EC.[46,47] Meroni *et al.* demonstrated that the presence of statins during incubation of human umbilical vein endothelial cells with antiphospholipid antibodies prevented EC activation *in vitro*.[48] In addition, the potentially beneficial effects of statins have also been demonstrated *in vivo*. After six months of therapy with statins patients showed a significant increase in coronary flow reserve and maximum coronary flow using pharmacological stress with dypridamole.[49] Also in patients with essential hypertension, long-term, effective blood-pressure reduction resulted in improvement in the impaired FMD of the brachial artery.[50] These results are promising. Whether similar effects will be reached in patients with systemic autoimmune disease and the increased risk profile for developing cardiovascular disease can be changed beneficially has to be demonstrated.

CONCLUSION

In summary, we propose that systemic autoimmune diseases and systemic vasculitis are associated with EC activation, during active disease in particular. This EC activation *in vivo* is related to parameters of EC dysfunction. It is suggested that chronic EC dysfunction in patients with systemic autoimmune diseases and systemic vasculitis will prove to be an independent additional risk factor that contributes to the development of atherosclerosis. The presence of EC dysfunction could explain the increased prevalence of premature atherosclerosis found in these patients. For clinicians it is important to realise that it seems possible to influence EC dysfunction and endothelium-dependent vasodilation. Randomised, controlled, intervention studies in patients with systemic autoimmune disease aiming at a reduction of the cardiovascular risk profile, including decrease in EC dysfunction, and development of premature atherosclerosis are eagerly awaited.

NOTE

This article is based on a contribution at the symposium 'Treatment of systemic autoimmune diseases', 14 february 2003, Groningen.

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REVIEW

New developments in staging and follow-up of patients with Hodgkin's lymphoma

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ABSTRACT

Adequate staging of newly diagnosed patients with Hodgkin's lymphoma enables optimal treatment planning, which is of particular importance for finding a balance between treatment efficacy and toxicity. In this review an overview is given of the current knowledge on initial staging and the role of imaging modalities during and after treatment. A promising new tool is whole-body positron emission tomograpy (PET) scanning with fluorodeoxyglucose (FDG). This modality is particularly useful for the evaluation of a residual mass at the end of treatment and for the assessment of the prognostically relevant 'early response' to chemotherapy. Although high survival rates have been reported for patients treated within the context of clinical trials these rates are generally lower in population-based series. In the general population comorbidity is present in half of all elderly Hodgkin patients. Since comorbidity has great impact on treatment planning and survival, this prognostic factor should be taken into account in future trials.

DIAGNOSIS AND HISTOLOGY

In 80 to 90% of the patients the first manifestation of Hodgkin's lymphoma (HL) is lymphadenopathy, most frequently located in the neck. For diagnosis an excisional lymph node biopsy is required to fully appreciate the architecture of the lymph node, in which the relative proportion of reactive bystander cells and malignant Hodgkin/Reed Sternberg cells (H/RS cells) can vary widely. The former histological classification (the Rye classification) recognised four subtypes: lymphocyte predominant (LP), mixed cellularity (MC), nodular sclerosis (NS) and lymphocyte depletion (LD).[1] The Revised European-American classification of malignant lymphomas (REAL classification) included some modifications, which have been adopted by the recent World Health Organisation (WHO) classification.[2] The nodular lymphocyte predominance type of Hodgkin's lymphoma is now considered to be a separate entity because of its distinct histological and clinical features with a prolonged indolent clinical course (*table 1*).[3]

The cellular composition of the neoplastic nodules varies widely among patients with the nodular sclerosis subtype. This prompted the British National Lymphoma Investigation (BNLI) group to propose a histological grading based on the number of H/RS cells and amount of fibrosis, which appeared to be of prognostic value.[4] Several groups[5,6] confirmed this prognostic significance, although others reported no survival differences.[7] In a recent population-based study this grading lost its independent prognostic value with the more frequent use of chemotherapy eradicating occult abdominal disease, which is more prevalent in the group with the worst prognostic.[8]

Table 1
Histological classifications of Hodgkin's lymphoma

RYE CLASSIFICATION	REAL/WHO CLASSIFICATION
Lymphocyte predominance	Nodular lymphocyte predominance
	Classical Hodgkin's lymphoma
Nodular sclerosis	Nodular sclerosis (*grades 1 and 2*)
Mixed cellularity	Mixed cellularity
Lymphocyte depletion	Lymphocyte depletion
	Lymphocyte-rich

STAGING

Staging classifications

Staging is necessary to determine the location and extent of disease, to define evaluable manifestations and prognostic factors and is the hallmark for the choice of treatment. Furthermore, staging allows comparison of treatment results between different study groups.

The Ann Arbor classification was modified at the Cotswold meeting in 1988, driven by the greater appreciation of the prognostic significance of tumour burden and the increased use of CT scanning (*table 2*).[9,10] In essence the staging is based on the number of sites of lymph node involvement, whether lymph nodes are involved on both sides of the diaphragm, whether there is visceral involvement and whether B symptoms are present.[11]

Conventional work-up

The initial work-up includes a complete history, physical examination, laboratory investigations and radiological examination. Thoracic CT scanning is useful as it has a considerable potential to influence the initial treatment policy. Staging below the diaphragm is hampered by false-negative results of CT scanning due to inability to detect HL in normal sized nodes and the difficulties in detecting HL in the spleen by CT scanning or ultrasound.[12]

Although bone marrow involvement is relatively uncommon a bone marrow biopsy is recommended, because of the high impact of a positive bone marrow on treatment planning.

Lymphangiography has nearly vanished from the work-up, not because of its diagnostic value but mainly because it is invasive, it requires great skill on the part of the radiologist, it has a prolonged examination time and is poorly rewarding economically.

Nowadays, staging laparotomy is rarely performed because of the lack of survival benefit, since the use of certain clinical criteria have made it easier to define patients likely to have occult abdominal disease.[13]

Gallium-67 scintigraphy is not believed to be accurate for the initial staging of Hodgkin patients. However, a pretreatment gallium scan is useful in the assessment of residual radiographic abnormalities after treatment, as a negative mass after treatment is likely to represent fibrosis if it was positive before.[14]

At presentation, most patients (70 to 80%) have stage II or III, whereas 10 to 15% have either stage I or stage IV disease.

New imaging modalities for staging

Magnetic resonance imaging (MRI) appears to be sensitive for the evaluation of bone and/or bone marrow involvement. However, this modality has the disadvantage that only a limited area of the body can be investigated, and therefore is used for evaluation of clinically suspected areas.[15]

The diagnostic yield of somatostatin receptor scintigraphy has been reported to be inferior and this modality is not performed routinely.[16]

Table 2

Staging notation of Hodgkin's lymphoma according to the Cotswold-modified Ann Arbor classification

Stage I	Involvement of a single lymph node region or lymphoid structure (spleen, thymus, Waldeyer's ring)
Stage II	Involvement of two or more lymph node regions on the same side of the diaphragm (the mediastinum is a single site, whereas hilar lymph nodes are considered bilaterally). The number of anatomical sites is indicated by a subscript (e.g. II_3)
Stage III	Involvement of lymph node regions or structures on both sides of the diaphragm III_1 With splenic, hilar, celiac or portal nodes III_2 With para-aortic, iliac, mesenteric nodes
Stage IV	Diffuse or disseminated involvement of one or more extranodal organs or tissue, with or without associated lymph node involvement
DESIGNATIONS APPLICABLE TO ANY DISEASE STAGE	
A	*No systemic symptoms*
B	*B symptoms present, one or more:* *– Unexplained weight loss >10% during previous six months* *– Unexplained fever (>38°C) during the previous months* *– Recurrent drenching night sweats during the previous months* *B symptoms generally correlate with advanced stage and bulk disease. Fever and weight loss have more negative prognostic impact than drenching night sweats*
X	*Bulky disease is present when:* *– A palpable lymph node defined by the largest dimension is >10 cm* *– The maximum width of a mediastinal mass is > one-third of the internal transverse diameter of the thorax at the level of T5/6 interspace on a chest X-ray*
E	*The subscript 'E' is used for documented limited extranodal extension contiguous or proximal to the known nodal site. More extensive extranodal disease is designated stage IV*
CS	*Clinical stage*
PS	*Pathological stage (as determined by laparotomy)*

Positron emission tomography (PET) with fluorodeoxyglucose (FDG) is a very promising noninvasive modality for the staging of Hodgkin patients. With whole-body FDG-PET, tomograpic images can be generated of the entire patient, displaying increased cellular glucose uptake and metabolism enabling the detection of disease, also in nonenlarged nodes/organs. Whole-body PET has been reported to be at least as sensitive as conventional staging procedures for initial staging,[17,18] being particularly useful for detection of extra-nodal localisations.[19,20] However, additional PET scanning in a study of 33 consecutive Hodgkin patients[21] did not influence planned treatment strategies based on conventional staging.
Although FDG-PET is still relatively scarce in the Netherlands hampering its routine use in staging of Hodgkin patients, its availability is growing. The high absolute costs of FDG-PET have to be put in the perspective of the better diagnostic yield and, more important, on the impact on patient management (*table 3*).

Elderly patients and comorbidity
For patients with other cancers it has been demonstrated that the presence of comorbidity is associated with less aggressive treatment and impaired survival.[22]
Comorbidity can influence therapeutic decision-making, since it might be a contraindication for anticancer treatment or a reason for dose reduction. The reduced survival rates for patients with comorbidity might also be related to a higher rate of treatment-related complications or to the increased risk of death caused by the comorbid condition itself. Experimental studies may use comorbidity as one of their restrictive eligibility criteria, subsequently underestimating its prevalence and relevance.
The prevalence of comorbidity appeared to be more than 50% in Hodgkin patients >60 years in a population-based study in the southeast of the Netherlands. Comorbidity was associated with a 50% reduction in the application of chemotherapy and with impaired overall survival. Whether this policy is justified or not deserves further investigation, since with increasing life expectancy of the European population this issue will be of growing concern.[23] Comorbidity is probably one of the major reasons for the lower overall survival rates in the general healthcare environment compared with those reported by clinical trials or referral centres.[24] Since comorbidity has great impact on treatment planning and survival, this prognostic factor should be taken into account in future clinical trials.

TREATMENT SELECTION

Early stage
Selection of initial treatment for Hodgkin's disease is based on the stage at presentation and prognostic factors.[25] In the past, radiotherapy was only the treatment for early stage (CS I-II) disease. Nowadays there is a trend to minimise late treatment-related complications with the use of lower doses of radiotherapy with smaller fields and with more frequent application of chemotherapy to treat occult (abdominal) disease.[26] This philosophy is underscored by the result of a recent meta-analysis demonstrating that adjuvant chemotherapy in early stages halved the ten-year risk of treatment failure compared with adjuvant radiotherapy.[27] The European Organisation for Research and Treatment of Cancer (EORTC) subdivides patients with early stage Hodgkin's lymphoma in either '*favourable*' or '*unfavourable*' by using a set of clinical criteria, corresponding with the risk of undetected abdominal disease. Patients are considered 'unfavourable' if they had any of the following: an ESR >50 mm/h, an ESR >30 mm/h in the presence of B symptoms, a mediastinal mass with tumour/thorax ratio of >0.35 (see *table 2*), or four or more sites of disease. Phase III clinical trials are ongoing to prove that unfavourable patients will profit from more intensive treatment.[28]

Advanced stage
Patients with advanced stage (CS III-IV) should be treated with chemotherapy. The ABVD (adriamycin, bleomycin, vincristine, dacarbazine) regimen is at least as effective as MOPP (mechlorethamine, vincristine, procarbazine,

Table 3
Value of the use of several imaging modalities for initial staging, assessment of early response, restaging at end-of-treatment and for evaluation of a residual mass in patients with Hodgkin's lymphoma

SCAN	INITIAL STAGING	EARLY RESPONSE	END-OF-TREATMENT	RESIDUAL MASS
CT	Mandatory	Routine in trials (after 3-4 cycles)	Mandatory	CT scans with several months interval
PET	Sensitive for extranodal disease Probably not changing policy	Promising as prognosticator	Promising as prognosticator	Promising to rule out active disease
MRI	Useful for evaluation suspected bone-marrow involvement	–	–	–
Gallium	Useful for comparison in case of residual mass after treatment	–	–	Negative scan likely reflects fibrosis, if positive before

prednisone)/ABV and superior to MOPP alone.[25,29] In a recent update of a randomised trial comparing ABVD with the MOPP/ABV hybrid regimen the overall and disease-free survival were similar for both patient groups.[30] However, ABVD has less germ cell and haematopoietic stem-cell toxicity and for this reason ABVD is considered to be the treatment of choice for patients with advanced Hodgkin's lymphoma.[25,29,30] The German Hodgkin Study Group has introduced the BEACOPP (bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, prednisone) regimen. In this regimen the drug etoposide is introduced, the dose intensity is increased (especially in the escalated regimen) and the most cytotoxic drugs (doxorubicin, cyclophosphamide, and etoposide) are given early in each cycle. In the HD9 trial patients with advanced disease were treated with either COPP (cyclophosphamide, vincristine, procarbazine, prednisone)/ABVD (the former German standard regimen) or with baseline BEACOPP or with increased-dose BEACOPP. The COPP/ABVD arm was stopped prematurely because increased BEACOPP resulted in better tumour control and overall survival.[31] Whether BEACOPP leads to superior cure rates over ABVD without endangering the initial therapeutic win by a long-term loss caused by treatment-related sequelae remains a matter of debate and will be studied in a recently opened study for patients with advanced Hodgkin's lymphoma with the highest risk of treatment failure (International Prognostic Score of at least 3, see *table 4*).
The value of additional radiotherapy for patients with advanced disease who have achieved complete remission on treatment with chemotherapy is debatable. In a recent EORTC study no survival difference was reported for patients treated with or without involved field radiotherapy after achievement of complete remission on six to eight cycles of MOPP/ABV.[33] This is in line with a recent meta-analysis reporting better overall survival for patients who received additional cycles of chemotherapy as compared with patients who received additional radiotherapy following initial chemotherapy. This survival advantage was due to lower treatment-related late mortality for patients who only received chemotherapy.[34]
All newly diagnosed Hodgkin patients should, when possible, be treated in the context of a prospective clinical trial since this is the only way to improve survival for future patients.

MONITORING

Monitoring during treatment

History and physical examination during treatment should be directed towards B symptoms, toxicity and the reduction of involved sites, since chemotherapy should be changed in case of failure to respond. Laboratory testing is needed for follow-up of abnormal results and for dose scheduling. Chest radiographs are useful to monitor intrathoracic sites and to exclude infections or toxicity.
Patients with disease visible only on CT scan should be scanned halfway through chemotherapy. Such documentation is useful for evaluating the rate of response, since *early complete response* predicts superior overall and disease-free survival. Whether this should have impact on treatment planning (number of cycles, for example) is addressed in clinical trials.[35] PET scanning appears to be a promising tool for the assessment of this early complete response. A predictive value for lower risk for relapse has been reported when the PET scan is negative after three cycles[36] or even after one cycle of chemotherapy (*table 4*).[37]

Table 4
The International Prognostic Score (IPS) for patients with advanced Hodgkin's lymphoma includes seven unfavourable features at diagnosis[32]

FACTOR	UNFAVOURABLE
Serum albumin	<40 gram/l
Haemoglobin	<10.5 g/dl
Gender	Male
Age	>45 years
Stage	IV
Leucocyte count	$>15*10^9$/l
Lymphocyte count	$<0.6*10^9$/l and/or <8% of leucocyte count

This analysis showed a continuous decline in the five-year event-free survival with increasing number of clinical risk factors present.[32]

NUMBER OF UNFAVOURABLE FACTORS	FIVE-YEAR EVENT FREE SURVIVAL (%)
None	84
1	77
2	67
3	60
4	51
5 or more	42

Evaluation after completion of treatment (restaging)

About one to two months following the completion of therapy the response should be documented by history, clinical examination and imaging.
CT scanning is much more sensitive than the chest X-ray for assessment of complete remission in the chest.[38] Residual abnormality seen on CT must be evaluated to distinguish between residual fibrosis and active disease.[39] This distinction can be made by serial studies, since benign disease will remain stable or decrease, while persistent Hodgkin's disease will increase in size. Persistent and unexplained elevation of the ESR is an indication for close surveillance.[40]

The value of scintigraphy with 67-gallium lies primarily in assessing the results at the end of treatment and not at initial diagnosis. In the evaluation of residual disease is 67-gallium scanning particularly useful, because it helps to differentiate between active tumour tissue and fibrosis. A residual mass that is gallium negative usually represents fibrosis, and follow-up without therapy may be warranted.[41] For this purpose a pretreatment gallium scan is useful for comparison.

Of special interest is the promising role of PET scanning in restaging. In a study of residual post-treatment masses in 58 patients, the negative predictive value of PET scanning was 100%, whereas the significance of a positive scan was less certain.[42] In another study a negative PET scan did not exclude residual disease completely, although progressive disease was more consistently associated with a positive PET scan compared with residual masses found with CT scan.[43] In a study of 81 patients the accuracy of PET appeared to be superior (91% for PET *versus* 62% for conventional imaging) for restaging using biopsy and/or clinical follow-up as confirmation.[44] Since a PET scan can be false-positive at the end of treatment, for example due to residual inflammation, a pretherapy PET scan is advisable for comparison.

End-of-treatment PET scanning appears to have prognostic significance. In 60 patients who underwent end-of-treatment PET scanning the two-year progression-free survival was 91% for PET-negative *versus* 0% for PET-positive patients. Moreover, PET scanning was the first tool that became positive for relapse.[45] In another study, relapse was more frequent in patients with a PET-positive (6/10) *versus* a PET-negative residual mass (3/19).[46] Whether PET scanning will allow for intensified treatment and possible cure of more patients as well as the potential economic advantages has yet to be demonstrated.

Late complications after treatment

Awareness of treatment-related sequelae is important and deserves special attention during follow-up.[25] The most important sequelae are briefly mentioned here.

Hypothyroidism develops gradually after mantle field irradiation, with a 20-year cumulative incidence of up to 41%.[47]

The increased risk of coronary artery disease following irradiation to the mediastinum should be reduced by the use of modern radiation techniques.[48]

Pneumonitis and pulmonary fibrosis is a common complication following mantle field irradiation and can be potentiated by bleomycin.[49]

The chance of maintaining fertility is greater among females than males and cryopreservation of semen prior to treatment should be considered in each patient.[47]

Secondary acute myeloid leukaemia often presents initially as myelodysplastic syndrome and is frequently refractory to treatment. The cumulative risk tends to plateau after 10 to 15 years and is associated with drugs as mechlorethamine and procarbazine.[50]

Secondary non-Hodgkin's lymphoma develops 5 to 15 years post-treatment with a cumulative incidence of up to 4 to 5%.[50] The incidence of secondary solid tumours continues to increase with prolonged follow-up. The most common tumours are of the lung, female breast, stomach, thyroid and bone, frequently localising within the previous irradiated field.[50,51]

Long-term follow-up after treatment

Following restaging after the completion of therapy, patients should be seen at regular intervals. General history, physical examination, ESR, complete differential blood count (to screen for bone marrow dysfunction), alkaline phosphatase, gGT, LDH, serum albumin and a chest X-ray (particularly in smokers treated with radiation therapy) is recommended at each visit. Testing of thyroid function at least once a year is recommended after mantle irradiation. Annual mammography should be performed five to ten years after treatment in women treated with mantle irradiation, especially when treated at a young age.

The site of relapse is partially determined by the type of initial therapy given. The use of the proper radiation technique should achieve an in-field disease control rate of more than 96%. The appropriate use and frequency of imaging such as CT scanning in the routine follow-up is not clear, since retrospective studies have shown that the majority of relapses are detected from the evaluation of symptoms rather than routine examination or imaging studies.[52] Moreover, it is not clear whether earlier detection of relapse with routine imaging will have other implications on the outcome of retreatment other than providing lead-time.

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The effect of granisetron, a 5-HT3 receptor antagonist, in the treatment of chronic fatigue syndrome patients – a pilot study

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ABSTRACT

Objective: To explore the effect of granisetron, a 5-HT3 antagonist, on fatigue and functional impairment in patients with chronic fatigue syndrome (CFS).

Methods: Five female patients were eligible to receive oral granisetron for one month (1 mg a day for the first two weeks and 2 mg a day for the second two weeks). The patients had to be between 18 and 65 years of age and suffering from CFS according to the CDC criteria. The effect was assessed by pre- and post-testing, using validated instruments designed to assess the different dimensions of CFS. Treatment response was also evaluated by visual analogue scales (VAS) for fatigue. Analysis was based on intention to treat.

Results: Treatment with granisetron resulted in significant improvement in fatigue severity and functional impairment. Activity level showed no significant increase.

Conclusion: The promising results of this study have encouraged us to perform a placebo-controlled, double-blind study to evaluate the efficacy of 5-HT3 receptor antagonists in the treatment of CFS.

INTRODUCTION

Chronic fatigue syndrome (CFS) is a medically unexplained syndrome, characterised by severe disabling fatigue for a period of at least six months, which has led to considerable impairment in daily functioning.[1] Various accompanying symptoms may be present, such as headache, joint and muscle pain, sore throat, and impaired memory and concentration. Of the many therapeutic interventions that have been undertaken so far, only cognitive behaviour therapy (CBT) and graded exercise therapy (GET) have met with succes.[2,3]

There is accumulating data in the literature supporting an important role for serotonin (5-hydroxytryptamine) in the neurobiology of CFS. Neuropharmacological studies point to an upregulated serotonin system.[4-7]
In a randomised controlled trial by our own research group, the selective serotonin reuptake inhibitor (SSRI) fluoxetine proved to be ineffective in CDC-diagnosed CFS subjects for the treatment of fatigue and depression,[8] which is also in line with upregulation of the serotonin system. Positive reports of the use of serotonin inhibitors in the treatment of patients with fatigue (due to chronic hepatitis[9] and to fibromyalgia[10,11]) support an effect. Based on these findings, we hypothesise that a serotonin antagonist could be effective in CFS. Therefore, we undertook this pilot study.

MATERIALS AND METHODS

Patients

Five female CDC-diagnosed patients with a high fatigue level and a substantial impairment in daily life, reflected by the Checklist Individual Strength (CIS)[12,13] and the

**J.W.M. van der Meer was not involved in the handling and review process of this paper.

Sickness Impact Profile-8 (SIP-8),[14,15] were treated with granisetron. The cut-off point of the CIS fatigue severity subscale was set at 40 and the weighted total score of the SIP-8 was set at 800.

In a CBT multicentre, randomised controlled trial by our research group, the CBT treatment protocol did not seem to be suitable for a group of CFS patients with low activity patterns.[2] Therefore, we selected patients with a low activity pattern. Patients whose average daily physical activity scores stayed below the reference score in at least nine of the twelve assessment days could be included.[16] The activity level was assessed prior to the treatment period with an actometer. We chose only female subjects, because in CFS the ratio male/female is approximately 1:4[17] and combined with low activity as a disease characteristic, we created a homogeneous group.

Additional criteria were patients aged between 18 and 65 years, and no previous or current engagement in CFS research. Pregnant or lactating women and patients who were taking psychotropic medications were excluded. We received ethical clearance to perform a pilot study and obtained written informed consent from all patients.

Design and procedures

There were four evaluation moments (E1-E4): E1 at baseline, E2+E3, in the middle and at the end of the treatment period and E4 at follow-up, two weeks after the treatment period. The treatment period was divided in two periods of two weeks. During the first period, the patients received an oral dose of granisetron of 1 mg a day. After two weeks the effect, compliance and side effects were evaluated. If the evaluation showed no significant improvement, the dose was increased to 2 mg a day.

Analysis was based on intention to treat. A linear model for repeated measures was used to analyse the effect of granisetron on the outcome measures CIS fatigue severity, CIS activity and SIP-8.
The four evaluation moments were analysed as well as the three evaluation moments during the medication period. The visual analogue scales (VAS) actual fatigue scores were analysed by the Wilcoxon signed-rank test.

ASSESSMENTS

Fatigue severity

The Checklist Individual Strength (CIS) is a reliable and validated self-report questionnaire. We used the subscale fatigue severity of the CIS (CIS fatigue severity).[12,13] The score on this eight-item scale ranges from 8 (no fatigue) to 56 (maximally fatigued).

CIS fatigue severity analysis during the medication period (E1-E3) calculated a significant decline in time (p=0.046). The significant drop during the medication period (E1-E3) means that patients reported significantly lower fatigue levels after treatment with granisetron. Analysis over four measurements is significant in time as well (p=0.026). Follow-up (E4) showed an increase in fatigue severity after discontinuation of granisetron (*figure 1*).

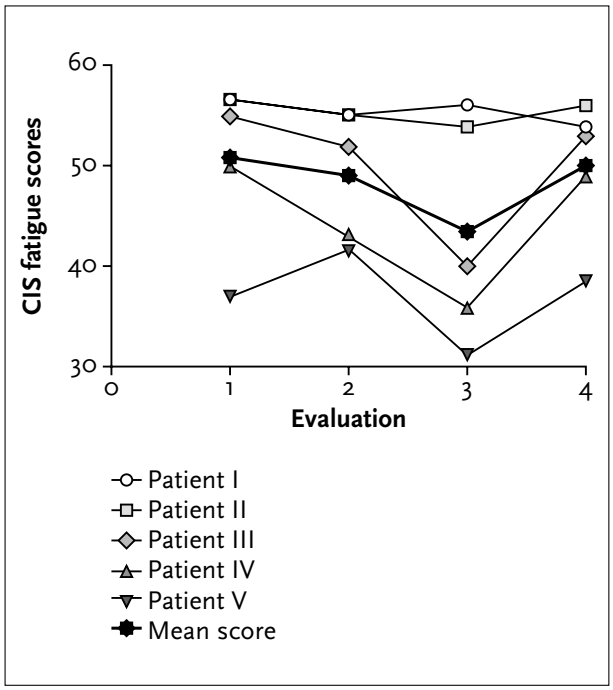

Figure 1
Effect of granisetron in five patients on the outcome variable fatigue severity (CIS fatigue severity)

Visual analogue scales (VAS) (100 mm) are used to determine the actual fatigue severity. VAS ratings were used to evaluate the one-month treatment period. VAS assessments took place during the medication period (E1, E2 and E3).
The VAS actual fatigue showed a significant drop of 29% in the mean fatigue scores (p=0.042) during the treatment period (*table 1*).

Table 1
Effect of granisetron in five patients on the outcome variable VAS actual fatigue severity (p=0.042)

TREATMENT	VAS MEAN	SD	SEM	RANGE MM
E1 – actual	76.4	13.7	6.1	59-95
E2 – actual	65.2	21.4	9.6	34-93
E3 – actual	54.4	28.1	12.6	22-92

Functional impairment

The sickness impact profile (SIP-8) measures the influence of symptoms on daily functioning, using the following eight subscales to rate both physical and psychological disability: home management, mobility, alertness behaviour, sleep/rest, ambulation, social interactions, work and recreation, and pastimes.[14,15]

SIP-8 analysis during the medication period (E1-E3) showed a significant decline in time (p=0.008). Patients reported significantly less functional impairment during the one-month medication period. Analysis over four measurements is significant in time as well (p=0.005). Follow-up (E4) showed an increase in functional impairment after discontinuation of granisetron. Within two weeks the mean SIP-8 score returned to the baseline level (*figure 2*).

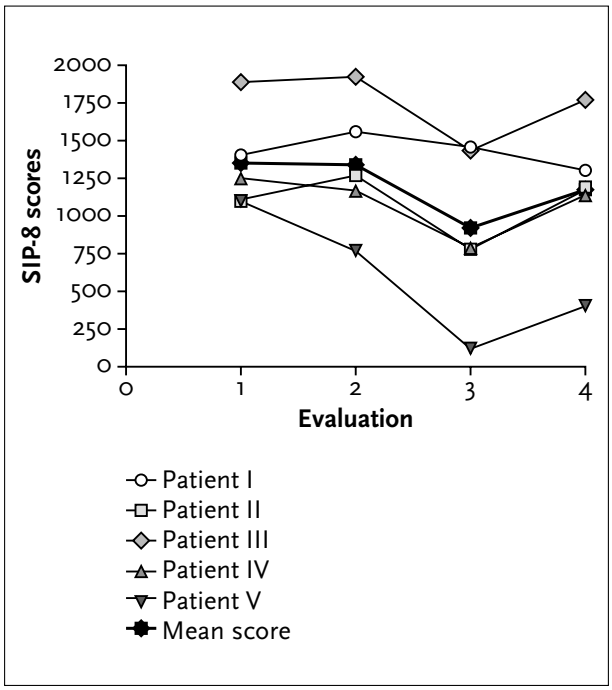

Figure 2
Effect of granisetron in five patients on the outcome variable functional impairment (SIP-8)

Activity level

The actometer is a small motion-sensing device that can register and quantify human physical activity.[18] Three different activity patterns can be distinguished for patients with CFS: pervasively passive, fluctuating active and pervasively active. In an earlier study identifying physical activity patterns in CFS patients, we observed that healthy controls do not fall in the category of pervasively passive. Patients wore the actometer day and night for a two-week period. After the two-week period the average scores over 12 days were computed.[16]

Before treatment the mean actometer score was 44.6 (SD 22.2). During the last two weeks of the treatment period the mean actometer score was 46.2 (SD 20.3). Granisetron did not significantly change the mean actometer score (p=0.16).

The subscale activity of the CIS was used (CIS activity). The score on this three-item scale ranges from 3 (no activity) to 21 (maximally activity level).[12,13]

Analysis of the CIS subscale activity showed no significant improvement during the medication period (p=0.16). Analysis over four measurements is not significant in time either (p=0.191).

RESULTS

The five women had a mean age of 34 years (range 23-44 years). Three of the five patients had a pervasively passive actometer pattern. Two patients had activity level scores lower than the mean CFS score for 9 out of the 12 days. All patients finished their study.

In the first two weeks the oral dose of 1 mg granisetron was well tolerated, but none of the patients showed significant improvement. In the second period all patients received 2 mg granisetron a day.

Four out of five patients reported marked improvement. One patient did not report any improvement on the outcome variables fatigue severity, activity level and functional impairment. Another patient complained of constipation as a side effect of granisetron during the last few days of the treatment.

DISCUSSION

In this pilot study we evaluated the effect of granisetron, a serotonin receptor antagonist, in chronic fatigue syndrome patients with low activity patterns. We found a substantial decrease in fatigue and functional impairment in four out of five patients as assessed by CIS fatigue severity, SIP-8 and visual analogue scale. That these changes in scores are clinically relevant can be deduced from our observations that these patients and their partners reported a remarkable improvement in fatigue and functional impairment at the end of the treatment period.

That granisetron, a serotonin antagonist, could have a favourable effect in CFS is not totally unexpected. First of all, there are reports in the literature pointing to a postsynaptic hyper-responsiveness in CFS.[4,5] Also the challenge test with buspirone, a 5-HT agonist and D-fenfluramine, a serotonin reuptake inhibitor, met with exaggerated prolactine responses in CFS patients, consistent with a postsynaptic serotonergic hyper-responsiveness in CFS.[6,7]

Second, there are reports in the literature pointing to a favourable effect of serotonin antagonist in fatigued patients. Jones reported a positive effect on fatigue in a 35-year-old woman with profound fatigue associated with chronic hepatitis-C when treated with a 5-HT3 receptor antagonist.[9] Positive results in fibromyalgia studies[10,11] also support a favourable effect of serotonin antagonists in the treatment of fatigue.
A remarkable finding is that within two weeks after discontinuation of granisetron, follow-up showed a marked increased mean fatigue level score and an increased functional impairment score. Within a few days granisetron is eliminated from the system (T1/2 elimination is 9-12 hours). The increase in symptoms within a short period after discontinuation of the medication supports the hypothesis that the intervention with granisetron is responsible for the reported improvement.
In this open study, a placebo effect cannot be excluded. However, in previous placebo-controlled studies[8,19] we have not encountered remarkable changes in fatigue severity and functional impairment in the placebo group. The actometer activity level showed no significant improvement. A possible explanation is that a reduced level of fatigue will not immediately lead to an increased physical walking activity level, which is measured by the actometer. It is possible that CFS patients have quite structured daily routines. A reduced level of fatigue will probably not immediately lead to a change in the daily routines. We treated patients with low activity patterns. In addition there may be deconditioning of the patients, which is not reversible in the short term of one month. It might take more than four weeks to change the rather static activity patterns, despite a decreased level of fatigue. Perhaps a longer treatment protocol and a longer registration period could lead to (significantly) improved actometer activity levels. However, we do not know whether wearing off effects occur with prolonged treatment.
It is striking that the outcome measures showed an improvement of CFS-related symptoms after two weeks (*figures 1* and *2*). It is not clear whether the reported improvement after two weeks can be explained by a dose-dependent effect. A possible explanation is that time is a key factor in the reported improvement. It is possible that granisetron induced a postsynaptic receptor modification in time or decreased the sensitivity of the postsynaptic receptors.
Data in the literature point in the direction that CFS and depression are opposing disease entities with contrasting neuroendocrine responses and 5-HT functioning.[20-22] The 'serotonin hypothesis' is the most widely accepted neural basis for depression. Drugs that preferentially increase serotonin activity by decreasing its reuptake (selective serotonin reuptake inhibitor, or SSRI) are effective in the treatment of depression[23-25] and not in the treatment of CFS, even when the patients are depressed.[8]
It is worth noting that there is a therapeutic delay of two weeks in the treatment of depression with a SSRI. It is conceivable that a similar delay occurs with 5-HT receptor antagonism as applied in the presented study.
It is remarkable that one patient did not respond. This 23-year-old woman with a CFS history of three years was no different from the other patients with regard to her history, CDC criteria, CIS fatigue severity score and SIP-8 score. Whether such nonresponsiveness has a serotonergic neuroendocrine basis has to be investigated in longer studies combined with serotonergic challenge tests and with serotonin receptor-status imaging studies. It is known that granisetron concentrations in blood may vary between subjects. A lower granisetron concentration might have caused the nonresponse in this individual. In this pilot study we did not measure blood granisetron concentrations. An interesting question is also whether further dose escalation would enhance the effect.

Our favourable results in this pilot study warrant a study with a randomised placebo-controlled, double-blind design. At the present time we are conducting such a randomised clinical trial with a longer treatment protocol and longer registration periods. In the future an interesting treatment concept might be a combination of a 5-HT3 receptor antagonist with CBT or GET.

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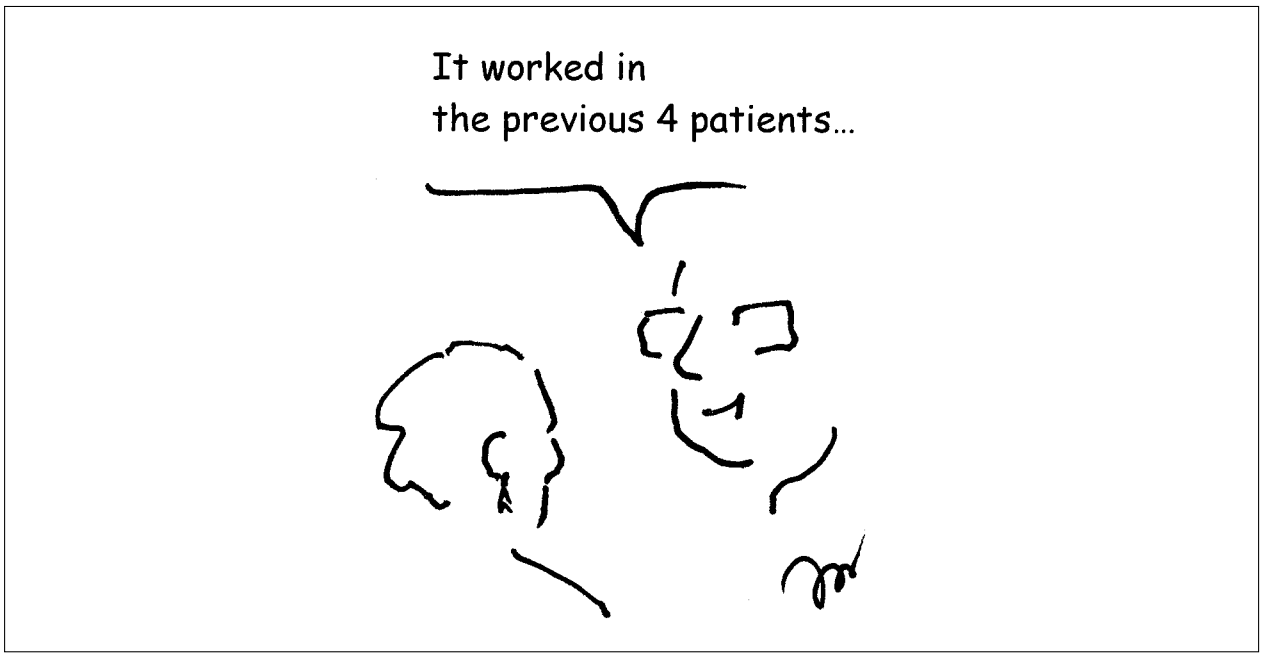

The, et al. Granisetron and chronic fatigue syndrome patients.

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A woman with a swollen, erythematous and tender thigh

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CASE REPORT

A previously healthy 50-year-old woman presented with a swollen, erythematous and very tender right thigh. She also noticed small and painful erythematous noduli on her left anterior shin and on both upper arms. She had symptoms of general malaise, loss of appetite, weight loss, fatigue, fever and night sweats. On examination the patient appeared ill and pale, with a temperature of 38.8°C. The small nodular lesions had the appearance of an erythema nodosum and on her right thigh large subcutaneous noduli were present (*figure 1*). During hospitalisation the eruption on the right thigh resolved spontaneously and similar lesions appeared on the left thigh (*figure 2*).
Laboratory examination revealed a markedly elevated erythrocyte sedimentation rate, anaemia, low albumin and liver enzyme abnormalities.

WHAT IS YOUR DIAGNOSIS?

See page 303 for the answer to this photo quiz.

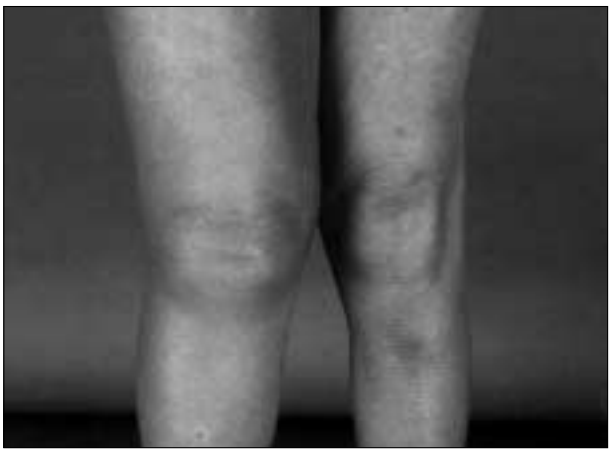

Figure 1
Swollen and erythematous right thigh at the moment of first presentation

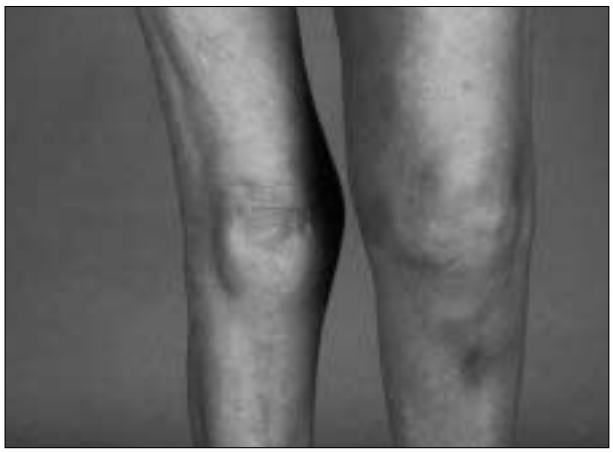

Figure 2
Swollen and erythematous left thigh six weeks later while in the meantime the swelling of the right thigh has disappeared

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

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ORIGINAL ARTICLE

Two Dutch families with hereditary hyperferritinaemia-cataract syndrome and heterozygosity for an HFE-related haemochromatosis gene mutation

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ABSTRACT

Hereditary haemochromatosis is an autosomal recessive disorder, leading to progressive iron overload, which is very common among the Caucasian population. In the vast majority of the cases, the hereditary iron overload is caused by mutations in the *HFE* gene. Most prominently this is the homozygous Cys282Tyr mutation. We report two Dutch families in which both propositi were found to be heterozygous for Cys282Tyr in the work-up of hyperferritinaemia. Frequent phlebotomies had no effect on the ferritin level, but led to microcytic anaemia. Finally, the family history with bilateral cataracts was the clue for the correct diagnosis.

Hereditary hyperferritinaemia-cataract syndrome (HHCS) is an autosomal dominant disease characterised by elevated serum ferritin levels and bilateral cataracts in the absence of iron overload. Several point mutations and deletions within the iron-responsive element (IRE) in the 5' noncoding region of the L-ferritin gene have been found in HHCS families. In the first Dutch family a G to C transition at position 32 was found and a G to A mutation at the same location was found in the second Dutch family.

In individuals with an isolated hyperferritinaemia (normal transferrin saturation), the presence of early onset (familial) cataract should raise the possibility of HHCS, even when Cys282Tyr heterozygosity is found.

INTRODUCTION

Hereditary hyperferritinaemia-cataract syndrome is a well-described disorder characterised by a combination of elevated levels of serum ferritin and early-onset bilateral cataracts.[1,2] Ferritins are heteropolymers of two different types of subunits (H-ferritin and L-ferritin), assembled to form a shell of 24 subunits with an internal core where iron can accumulate for storage purposes. The synthesis of both subunits is regulated by the interaction between a cytoplasmatic protein capable of binding iron, the so-called iron regulatory protein (IRP) and the highly conserved stem-loop motif, known as the iron responsive element (IRE) that is present at the 5' untranslated region (UTR) of the ferritin mRNA. When there is limited iron, the IRP binding to IRE inhibits the binding of the ferritin mRNA to ribosomes, and thus prevents the translation of the ferritin mRNA (see *figure 1*, also reviewed by Cazzola and Skoda).[3] Under conditions of abundant iron, the IRP cannot bind IRE because of the formation of 4Fe-4S clusters, leading to efficient synthesis of ferritin. In HHCS, mutations in the IRE make the L-ferritin mRNA translation independent of the iron status, since the IRP is not able to bind IRE.[2,4-9] HHCS is inherited in an autosomal dominant manner, although *de novo* mutations have also been reported.[10,11] The severity of cataract has been found to be related to the serum level of ferritin.[3,12] However, the location of the nucleotide substitution can lead to different levels of serum ferritin as well as to variable phenotype, even in individuals of the same family.[3,5,7]

Hereditary haemochromatosis is an autosomal recessive disorder characterised by an increased uptake of dietary

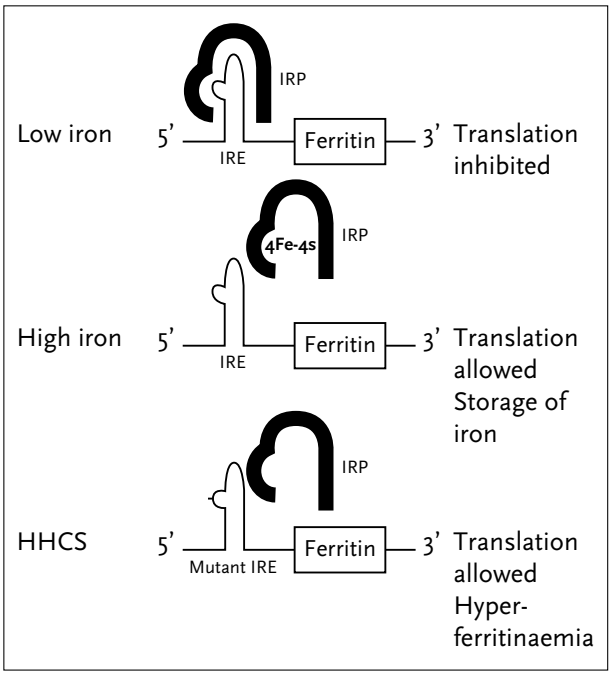

Figure 1

Schematic representation of the regulation of ferritin synthesis

This translational regulatory mechanism applies to the L- as well as the H-ferritin subunit. Upper panel: the situation when there is low iron, allowing the iron regulatory protein (IRP) to bind strongly to the iron responsive element (IRE) located at the 5' noncoding region of the ferritin gene. This prohibits the translation of the ferritin mRNA. Middle panel: when cellular iron is abundant, formation of 4Fe-4S clusters within IRP prevents binding to IRE, leading to efficient synthesis of ferritin.

Lower panel: the condition when the IRE is mutated in HHCS patients, meaning that irrespective of the iron status the IRP is not capable of binding IRE, allowing the translation and synthesis of ferritin, leading to hyperferritinaemia.

iron.[13,14] This eventually leads to iron loading in parenchymal organs, resulting in dysfunction of these organs, especially the liver, heart and pancreas. Two major mutations in the *HFE* gene have been described, Cys282Tyr and the His63Asp. Most haemochromatosis patients are homozygous for the Cys282Tyr mutation.[15] The role of the His63Asp mutation in iron overload is less clear, however. Even in the so-called compound heterozygotes (heterozygosity for both Cys282Tyr and His63Asp mutations) iron overload is usually mild and only present in 1 to 2% of the cases.[16] The iron overload can be treated by (repeated) phlebotomies.

We describe two heterozygous mutations in the IRE of the 5'-UTR of the L-ferritin gene co-inherited with a heterozygous mutation in the *HFE* gene. The co-inheritance of both mutations may be misleading, as is illustrated by our study.

PATIENTS AND METHODS

Family 1

A 53-year-old man was seen at our outpatient department after he had been investigated elsewhere. He was initially seen by his family doctor because of symptoms in his joints (backache and pain in the hips). The physical examination was unremarkable: body mass index (BMI) of 26.5 kg/m^2 and a normal blood pressure. Initial blood tests, performed elsewhere, showed a normal erythrocyte sedimentation rate, haemoglobin, leucocyte, and platelet counts, as well as normal liver and renal function. The glucose level was normal. The patient had a plasma cholesterol level of 5.9 mmol/l with normal triglycerides. Further analysis revealed a high serum ferritin level of 1750 μg/l (normal: 10-250 μg/l). Serum iron transferrin and transferrin saturation were normal. Despite the persistently normal values of the transferrin saturation, the patient was suspected of having HFE-related haemochromatosis. Genetic analysis of the *HFE* gene showed a heterozygosity for the G to A nucleotide substitution at position 845, responsible for the Cys282Tyr mutation (*figure 2A*). Thereafter, the patient was subjected to repeated phlebotomies, which had no effect on his ferritin level or his symptoms. On the contrary, the patient developed microcytic anaemia. Finally, the patient was referred to our hospital for further analysis of the hyperferritinaemia. The patient had an unremarkable medical history, except for operations because of bilateral cataracts. The first operation was when he was 27 years old (left eye), the second at the age of 38 (right eye). The family history was also positive for bilateral cataracts at a young age (see *figure 2A*). This prompted us to analyse the 5'-UTR of L-ferritin. The close relatives (first, second and third degree relatives) of the proband were tested for serum ferritin level, and those with a high ferritin level had undergone operations for bilateral cataracts. To confirm the phenotype of hereditary hyperferritinaemia-cataract syndrome, molecular analysis was performed.

Family 2

A 58-year-old man was found to have elevated serum ferritin level (1454 μg/l) during a routine examination of body iron status, with normal transferrin saturation. This patient was found to be heterogyzous for the Cys282Tyr mutation of the *HFE* gene (*figure 3A*). His BMI was 27.8 kg/m^2, he had a blood pressure of 140/80 mmHg, with a fasting plasma cholesterol level of 5.9 mmol/l, normal triglycerides, and a normal glucose level. His sister (BMI 29.9 kg/m^2, blood pressure of 130/75 mmHg, plasma cholesterol 6.3 mmol/l, normal triglycerides, normoglycaemic), who was also analysed because of hyperferritinaemia, was also found to be a carrier of the Cys282Tyr mutation, for which she was subjected to frequent phlebotomies, without any effect on the serum ferritin level. However, she developed

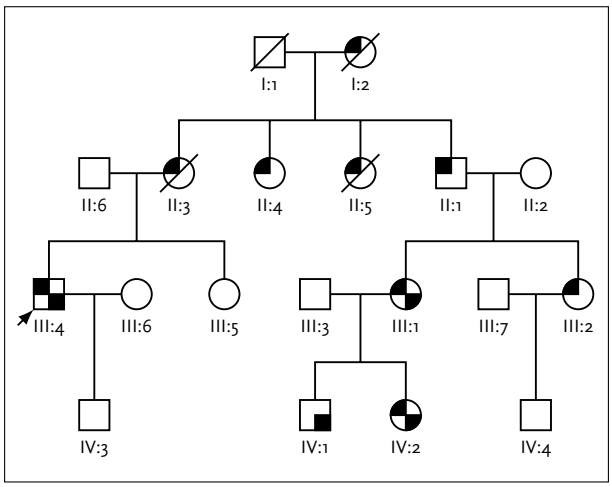

Figure 2A

Pedigree of the family 1 with the presence of cataract as well as heterozygosity for the Cys282Tyr mutation in the HFE gene

Cataract indicated by symbol with filled upper left quadrant, mutation in the *HFE* gene indicated by symbol with filled lower right quadrant, diagonal lines indicate deceased members and the proband is indicated by the arrow.

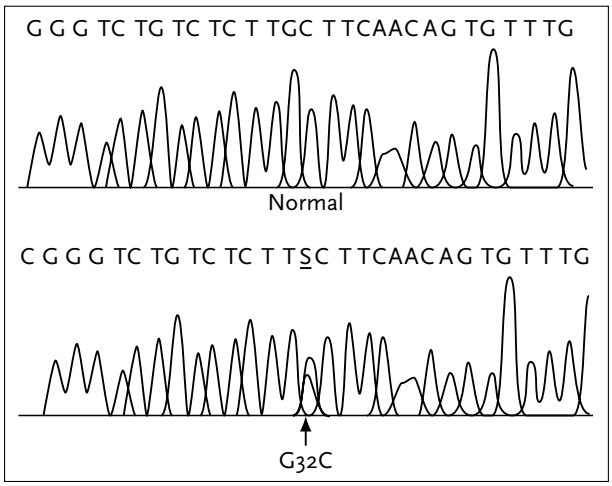

Figure 2B

Identification of the G to C mutation at position 32 in the IRE of the L-ferritin gene – sequence analysis of genomic DNA of a normal individual and of the proband

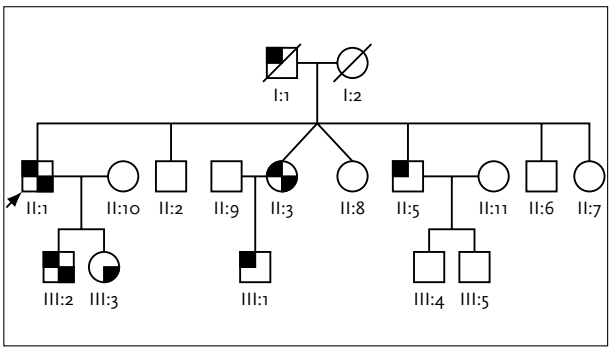

Figure 3A

Pedigree of the family 2 with the presence of premature bilateral cataracts as well as heterozygosity for the Cys282Tyr mutation in the HFE gene

Premature bilateral cataracts is indicated by symbol with filled upper left quadrant, Cys282Tyr mutation in the *HFE* gene is indicated by symbol with filled lower right quadrant, diagonal lines indicate deceased members and the proband is indicated by the arrow.

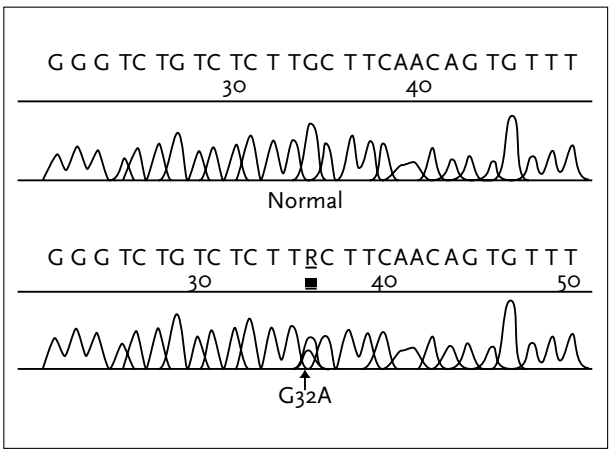

Figure 3B

Sequence analysis of genomic DNA of a normal individual and of the proband, showing the G to A mutation at position 32

microcytic anaemia. Both patients had a medical history of premature bilateral cataracts. The pedigree (*figure 3A*) shows the inheritance pattern of the Cys282Tyr mutation, as well as the family members with a history of premature bilateral cataracts necessitating surgery.

Molecular analysis of the 5'untranslated region of the L-ferritin gene genomic DNA was isolated from blood leucocytes according to standard methods. Primers were used to amplify the entire IRE of the L-ferritin gene.

Direct sequencing was carried out in both orientations of genomic DNA PCR products, performed on an ABI 3100 automated sequencer using a version 3 dye termination sequencing kit (Applied biosystems Torrence, CA, USA). Molecular analysis of the *HFE* gene for the G845A nucleotide substitution was performed based on the findings by Feder *et al.* and taking into account the 5569 G/A polymorphism in the choice of the nucleotide primers.[15]

RESULTS AND DISCUSSION

We found a heterozygous G to C transition at position 32 of the L-ferritin gene of proband 1 and the close relatives

(first, second and third degree) with high ferritin level and a medical history of surgery because of premature bilateral cataracts (*figure 2B*). The youngest member with bilateral cataracts was subjected to surgery at the age of seven, but she had already suffered from blurred vision at the age of four. The G32C substitution has previously been described in French and Italian pedigrees.[6,7,17] At the same position of the 5'-UTR of the L-ferritin gene, a G to A substitution was found in the second family (*figure 3B*). This mutation has also been reported previously in two Italian families.[5,8]

It is remarkable that the highly conserved guanine at position +32 is prone to mutations, and thus can be considered as a hot spot for mutations, since it has been found to be mutated in Italian, French and Dutch families.[5-8,17] The level of hyperferrritinaemia in the two Dutch families in this study is comparable with those previously described in the Italian and French families. However, as noted previously, there is a phenotypic diversity among subjects with the same mutation.[7] Because some of the described mutations in the IRE of L-ferritin in patients with HHCS are *de novo*, and in some patients with hyperferritinaemia the cataract is asymptomatic, this syndrome should be included in the differential diagnosis of hyperferritinaemia even in the absence of a positive family history. The exact mechanism causing hyperferritinaemia in HHCS subjects to lead to cataracts (and not to pathological conditions in other tissues/organs) remains unclear. Possible theories have been proposed. An abnormal deposition of L-ferritin (approximately tenfold accumulation of ferritin in HHCS patients, compared with normal individuals)[18] may cause alteration of local iron turnover, or modify other lens proteins, leading to oxidative lens damage.[19-21]

The next remarkable finding is the co-inheritance of G32C and G32A mutation of the L-ferritin gene with the Cys282Tyr mutation in the *HFE* gene in an heterozygous manner in both families in this study. Co-inheritance of alleles associated with HFE-related haemochromatosis and HHCS has been reported previously.[22] Heterozygous substitution of an A40G of the IRE of the L-ferritin gene together with His63Asp mutation of the *HFE* gene was found. However, the combination of mutations in two genes involved in iron metabolism, the Cys282Tyr in the *HFE* gene and mutation in the L-ferritin gene, have not been reported until now. Altogether, because of the high frequency of the Cys282Tyr mutation in a heterozygous state in persons from North-European descent (10% of the Caucasian population), the co-inheritance of this mutation with the mutation in the IRE of the L-ferritin gene as described in this study, could be regarded as coincidental. The propositus of both families were found to be heterozygous for the Cys282Tyr mutation of the *HFE* protein, meaning that *HFE*-related haemochromatosis was excluded in both propositi. However, another hereditary iron overload syndrome, primary haemochromatosis type 4 associated with mutations in the ferroportin-1 gene, remains possible.[23,24] This autosomal dominant disorder is characterised by relatively low serum iron level with an elevated serum ferritin level. One of the features in haemochromatosis type 4 is that the ferritin level increases prior to elevation of the transferrin saturation. Unlike the situation in HHCS, iron accumulates in reticuloendothelial cells. During phlebotomy (indicated in haemochromatosis type 4), haemoglobin as well as transferrin saturation may reach low levels despite high-normal serum ferritin. This finding may lead to confusion with HHCS (phlebotomy *not* indicated). In theory, there is a very small chance that in our patients the ferroportin-1 gene is also mutated, which can be investigated in future studies.

In 1997 Moirand *et al.* described a hepatic iron overload syndrome (also designated as dysmetabolic iron syndrome) characterised by hyperferritinaemia with a normal transferrin saturation.[25] The authors showed on the basis of HLA typing that this syndrome was not related to haemochromatosis. However, this syndrome was associated with various metabolic disorders; 72% of the patients (*vs* 35% of matched genetic haemochromatosis [GH] subjects) had a body-mass index of >25kg/m^2, 65% of the patients had hyperlipidaemia (*vs* 17% of GH subjects), 43% of the patients with an abnormal glucose metabolism (*vs* 8% of GH subjects), and 19% of the patients had hypertension (*vs* 12% of GH subjects).[25] Notably, these conditions are all components of the insulin-resistance syndrome. In our patients a clear association with the so-called dysmetabolic iron syndrome could not be demonstrated.

In conclusion, physicians should be aware that an isolated hyperferritinaemia (with a persistent normal transferrin saturation) does not per se imply that there is an iron overload and that the presence of an early onset cataract should raise the possibility of HHCS, even if there is a heterozygosity for a mutation in the *HFE* gene.

ACKNOWLEDGEMENT

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PTU-associated cutaneous vasculitis with ANCA anti-MPO and anti-PR3 antibodies

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ABSTRACT

A 36-year-old woman presented at our clinic with symmetrical, tender, palpable purpuric lesions on her lower legs and buttocks after restarting PTU therapy for relapsing Graves' disease. PTU-induced vasculitis was diagnosed with remarkable ANCA anti-MPO and anti-PR3 antibody positivity. The purpuric skin lesions resolved immediately after discontinuation of the drug and the ANCA titres lowered. In the presence of activated neutrophils, PTU could induce a high cytotoxity and injure the vessel walls. Treatment of choice is discontinuation of the drug. Sometimes more aggressive therapy as cyclophosphamide or plasmapheresis is warranted.

INTRODUCTION

Propylthiouracil (PTU) is a frequently used drug to treat hyperthyroidism. It inhibits the synthesis of thyroid hormones by competitive inhibition of the enzyme peroxidase. One of the most serious complications of this drug is PTU-induced vasculitis.
Antineutrophil cytoplasmic antibodies (ANCA) are important diagnostic markers associated with the spectrum of vasculitides that includes Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, and primary pauci-immune necrotising and crescentic glomerulonephritis.[1] Two specific types of ANCA have been shown to be useful in the diagnosis of this disease spectrum: antiproteinase 3 (anti-PR3) antibodies, which produce a cytoplasmic pattern of staining (c-ANCA) by indirect immunofluorescence, and antimyeloperoxidase (anti-MPO) antibodies, which produce a perinuclear pattern of staining (p-ANCA).[2] PTU may induce ANCA-positive vasculitis. The majority of these PTU-induced vasculitis cases are associated with anti-MPO ANCA.[1,3]
We describe a patient who presented with anti-MPO and anti-PR3 associated vasculitis while treated with PTU for Graves' disease.

CASE REPORT

A 36-year-old woman with Graves' disease was treated with methimazole for several months. Methimazole was withdrawn because of development of urticaria, and propylthiouracil (PTU) was started. The treatment was discontinued after one year of euthyreoidism. Nine years later the hyperthyroidism relapsed [free thyroxin 31 pmol/l (normal 11-25); thyroid-stimulating hormone <0.02 µ/l (normal 0.30-4.00)] and PTU treatment was restarted while waiting for I^{131} therapy. Several weeks thereafter she visited her general practitioner with symptoms compatible with sinusitis. Treatment was started with clarithromycin and loratadine. She had been treated likewise in the past without complications. A few days later the patient developed an erythematous rash. After withdrawal of the antibiotic treatment, the rash worsened and was accompanied by symmetrical, tender, palpable purpuric lesions on her lower legs and buttocks (*figures 1* and *2*). The patient was referred to our hospital. Laboratory test on admission revealed: ESR 33 mm/h (1-20), Hb 8.1 mmol/l (7.4-9.9), Ht 0.38 l/l (0.36-0.46), platelets $209*10^9$/l (150-400), white blood cells $2.9*10^9$/l (3.5-11.0). Differentiation (%): segmented cells 1 (40-75), lymphocytes 73 (20-45), monocytes 26 (1-12), no eosinophils, atypical lymphocytes ++. ANA and anti-dsDNA antibodies were negative.

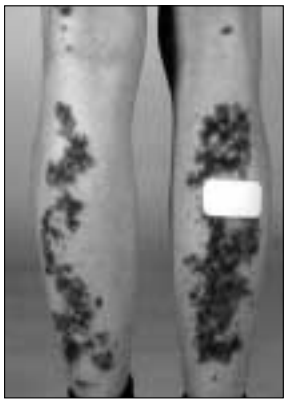

Figure 1
Cutaneous lesions of the lower legs of our patient (the plaster is there because of the biopsy taken from the lesion)

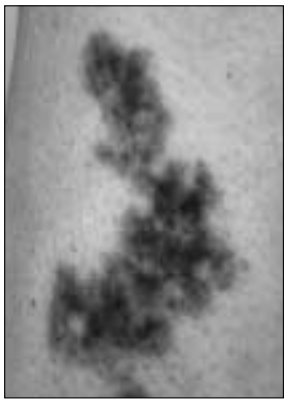

Figure 2
Detail of the cutaneous lesions

Immunofluorescence: p-ANCA positive; 1: >320. Specificity in Elisa: MPO and PR3 positive. Urine analysis showed no proteinuria, leucocyturia or erythrocyturia. Skin biopsy taken out of the rim of a palpable purpuric lesion was compatible with leucocytoclastic vasculitis (*figure 3*). Direct immunofluorescence studies were not performed. We diagnosed the patient as having PTU-induced vasculitis, and discontinued the drug. The skin lesions began to regress and resolved completely. The ESR returned to the normal range and the granulocytopenia resolved in five days. The ANCA (IFT) titre initially dropped to 1:160 after seven weeks of withdrawal although it rose again to 1:320 four months later, despite the good clinical condition of our patient.

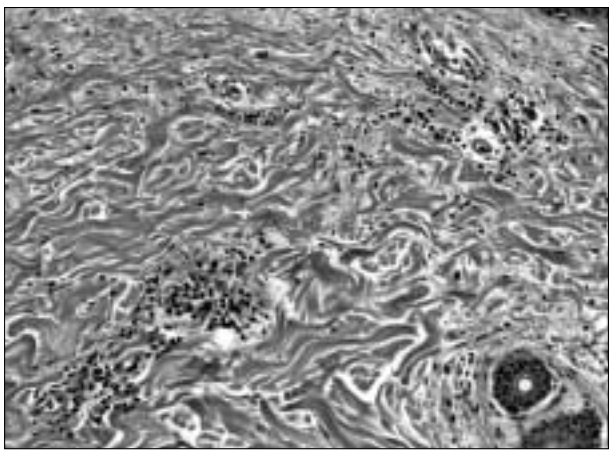

Figure 3
Detail of skin biopsy of leucocytoclastic vasculitis

DISCUSSION

Systemic complications of PTU treatment are mentioned in 1 to 5% of PTU users. The most common side effects are agranulocytosis, hepatotoxicity and drug-induced hypersensitivity.[4-7] Although PTU-induced vasculitis is well documented, it is a relatively rare side effect of PTU therapy. The association of positive ANCA with antithyroid drug therapy was first described by Dolman *et al.* in 1993.[8] In absolute amounts, more females than males are affected with PTU-induced ANCA positive vasculitis. Although in most cases the symptoms appear many months after starting the medication, some patients manifested symptoms within one month or even within two weeks after starting the drug.[4,5,8,9] Symptoms as fever, general fatigue, polyarthritis, myositis, scleritis, pleuritis, alveolar haemorrhage, pericarditis, nephritis, hepatitis and skin ulceration have been reported.[5,8,10-12] In some cases, the clinical features of PTU-induced vasculitis may be limited entirely to the skin, especially the breasts, the helices of the ears, the face and, as noted in our patient, the distal lower extremities.[5,9] After the medication is discontinued, the symptoms disappear in most cases.
Biopsy specimens of skin lesions generally show leucocytoclastic vasculitis. This is described as endothelial swelling, extravasation of erythrocytes and pronounced perivascular infiltration of neutrophils, with fragmented leucocytic nuclei in and around the vessels.[6,9] Some vessels are occluded by fibrin thrombi and some are even necrotic, as in our patient.[6,13] Most cases of PTU-induced vasculitis had negative direct immunofluorescence tests, but deposition of IgM, IgG, IgA, fibrinogen, and complement factor C3 in dermal blood vessel walls has been reported.[6,13]

In the literature only five patients, two with vasculitis of the skin, two with glomerulonephritis and one with arthritis, are described in whom both MPO-ANCA and PR3-ANCA were identified simultaneously.[8,11,12,14] More often the combination of MPO-ANCA and elastase-ANCA is found.[8,11] The mechanism of MPO-ANCA-associated vasculitis is still not clear. The altered state of self-tolerance present in these patients or an interaction between MPO and PTU resulting in an immunogenic compound may be responsible for development of the ANCA. Lam and co-workers found that PTU accumulation in the neutrophil seemed to be related to the oxidation of the medication, as there was an increased H_2O_2 accumulation in the cell during PTU uptake of these cells.[10] Others reported that the drug exhibited its high cytotoxicity in the presence of activated neutrophils, for instance neutrophils activated by an infection.[8,11,15] Following activation the neutrophils may release a large quantity of MPO and transform drugs to free radicals, resulting in vessel-wall injury.[15] Possibly a previous sensitisation to PTU could play a role in the

immunogenic spectrum as described by Wolf.[16] Indeed, our patient had no complications during previous use of this drug. The factors responsible for the production of anti-PR3 antibodies during PTU treatment are unclear. A puzzling fact in the case presented here is the combination of leucocytopenia and ANCA-induced vasculitis as described before by Dolman and Kitahara.[8,11] This seems to be in contradiction with the theories above describing the presence of activated neutrophils starting the vasculitis. Perhaps the neutrophilic activation is reflected in a shortage of circulating neutrophils as the neutrophils migrate to the vasculitis spots where the endothelial damage occurs. Furthermore, it has been reported that after activation the neutrophils may undergo an accelerated pattern of apoptosis, leading to cell death and further potential tissue damage secondary to necrosis.[17] But a bone marrow suppressive effect of PTU, as described by Balkin *et al.*,[18] could also be an explanation for the leucopenia, although to develop a vasculitis some neutrophilic activation, as described before, should accompany it. Caution in interpreting ANCA without knowing the medical history of a patient is mentioned by Cohen Tervaert, who described the presence of ANCA with specificity for MPO or PR3 in patients without vasculitis during treatment with antithyreoid drugs.[19] Gunton and Sera suggest alertness when using PTU in patients with ANCA as these ANCAs are usually not present at the start of the drug therapy.[20,21] Choi describes a patient who changed his ANCA pattern from c-ANCA to p-ANCA during treatment with PTU and back to a c-ANCA after the medication was discontinued.[22] Noh *et al.* investigated prospectively the development of MPO-ANCA in 73 patients treated with PTU for Graves' disease. They found development of MPO-ANCA in three of these patients. In two of them the increases were only temporary despite continuation of PTU therapy. The third patient developed oral ulcers, fever, diarrhoea and polyarthralgia after 17 months of PTU treatment. Her MPO-ANCA titre was highly increased. PTU treatment was discontinued after which the symptoms resolved and the MPO-ANCA titre decreased.[23] Whether the use of clarithromycin played a role in the development of vasculitis in our patient remains unclear. Clarithromycin itself may induce leucocytoclastic vasculitis, though very sporadically.[24-26] None of the clarithromycin-induced vasculitis cases mentioned leucocytopenia in the patients.

Although most patients (about 60% mentioned in case reports), like our patient, recover completely simply by withdrawal of PTU, some patients who have severe renal involvement or impairment of multiple organ systems may require high dosages of prednisone for several months.[4] Cyclophosphamide,[7,11,12] azathioprine,[7] naproxen,[4] or plasmapheresis[9,12,27] are other therapeutic treatments that have been used in isolated cases. Sequelae reported most often after resolving of vasculitis are scars resulting from necrosis of (sub)cutaneous tissue or arthralgia.[9] The ANCA usually returns to normal values, though sometimes ANCA titres persist for a longer time, as in this case.[7-9,12] Hyperthyroidism is treated either with radioactive iodine or with surgical treatment. Favouring the radioactive iodide treatment one should bear in mind that iodide may exaggerate symptoms of vasculitis even after withdrawal of PTU.[9] The increased movements of polymorphonuclear cells induced by the iodide into inflammatory sites are mentioned to be responsible for this reactivation of vasculitis.[28]

CONCLUSION

Propylthiouracil could be involved in inducing vasculitis. Recognition of the classic cutaneous features permits early diagnosis and may limit associated morbidity in these patients. The vasculitis is often prescribed as ANCA associated, though rarely both anti-MPO and anti-PR3 antibodies are involved. The exact mechanism by which PTU induces vasculitis remains to be resolved.

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CASE REPORT

Antinucleair antibody (ANA) positivity caused by paraneoplastic antibodies due to abundant p53 expression in early hepatic carcinoma

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ABSTRACT

A 51-year-old patient is described who presented with locomotor pain and highly significant positive ANA due to p53 antibodies, which appeared to be associated with primary hepatic carcinoma.

INTRODUCTION

In clinical oncology a diversity of tumour markers may be used, including α-fetoprotein (AFP) for hepatic carcinoma, carcino-embryonal antigen (CEA) for colorectal carcinoma and β-human chorion gonadotropin (β-hCG) for chorio-carcinoma. These markers are commonly applied in the follow-up of a specific tumour. For screening purposes, however, most of these markers cannot be used. In rheumatology antinuclear antibodies (ANA) may be used for screening purposes in several connective tissue diseases, particularly in systemic lupus erythematosus, but low ANA titres can be found in healthy individuals as well. ANA serology may become positive early in the course of some tumours due to tumour-associated antibodies. The patient presented here had a highly significant positive idiopathic ANA which turned out to be due to p53 antibodies. ANA may also be applied as a diagnostic marker in oncology.

CASE REPORT

A 51-year-old woman with no relevant medical history was seen at the Rheumatology Outpatient Department because of persistent locomotor pain which had started five years before. Consultation of an orthopaedic surgeon and neurologist did not deliver an explanation nor a diagnosis. The locomotor pain worsened during exercise. There were no signs of arthritis, synovitis nor fever. She complained of fatigue, loss of energy (about 30%) and photosensitivity of the skin when exposed to sunlight. Family history was negative for any disease. Physical examination revealed no abnormalities of the thorax and abdomen. The locomotor system revealed some degenerative joint disease, compatible with her age, and no symptoms suggestive of arthritis or synovitis. Five out of 18 tender points according to criteria set by the American College of Rheumatology (ACR) were positive. An additional chest X-ray was normal. Laboratory examination revealed the following: erythrocyte sedimentation rate (ESR) 8 mm/hr (normal: <12 mm/hr), C-reactive protein (CRP) 5 mg/l (normal: <10 mg/l), haemoglobin 8.2 mmol/l (normal: >7.2 mmol/l), with further unremarkable haematology parameters. Renal and liver function tests: ASAT 20 U/l (normal: <45 U/l), ALAT 27 U/l (normal: <45 U/L), alkaline phosphatase 57 U/l (normal: <105 U/l), gamma-glutamyltranspeptidase 66 U/l (normal <35 U/l). Iron saturation was 36% (normal 15-50%). Because anamnesis suggested photosensitivity, antinuclear antigens were determined which were highly positive (titre 1:320) with a speckled pattern but without known specificity for extractable nucleolar antigens (ENA) nor antibodies to DNA or centromeres.
In conclusion, this 51-year-old woman presented with degenerative joint disease and enthesopathy, photosensitivity and a speckled ANA, so far without known

specificity. The differential diagnosis of idiopathic ANA positivity primarily consists of neoplastic or lymphoproliferative disorders as no signs were found for autoimmune disease. Abdominal ultrasonography was requested as the biochemistry was normal. Ultrasonography of the liver revealed an intrahepatic process with a diameter of 5 cm, suggestive of a hepatocellular carcinoma. Additional laboratory investigation revealed a normal AFP: 3 kU/l (normal: <8 kU/l). The patient was sent to an internist/hepatologist for biopsy of the intrahepatic lesion and further investigations.

An extensive oncological work-up was carried out, including computerised tomography (CT) of the abdomen, which revealed a solitary highly vascularised tumour in the left lower lobe of the liver. No signs were found of liver cirrhosis. A biopsy of the intrahepatic lesion confirmed the suspected hepatocellular carcinoma with a diameter of 8 cm with nine satellite metastases (*figure 1*). The patient was sent for a hepatobiliairy procedure as the hepatic tumour appeared to be the primary process. Hemihepatectomy was performed aiming at a curative procedure.

Six months later the patient was seen again at the outpatient department because of progressive pain due to enthesopathy: 16 of the 18 tender points were positive. Serological investigation revealed a positive ANA (titre 1:160). Recurrence of the hepatocellular carcinoma was suspected. At a three-monthly ultrasonogram the tumour indeed appeared to have recurred at the resection site, which was confirmed by CT scanning. Dissemination investigation was negative. Then treatment was started with ablative radiofrequency, aiming at lysis of tumour cells due to heating. Further investigation of the initial highly positive ANA revealed that there were circulating p53 antibodies.

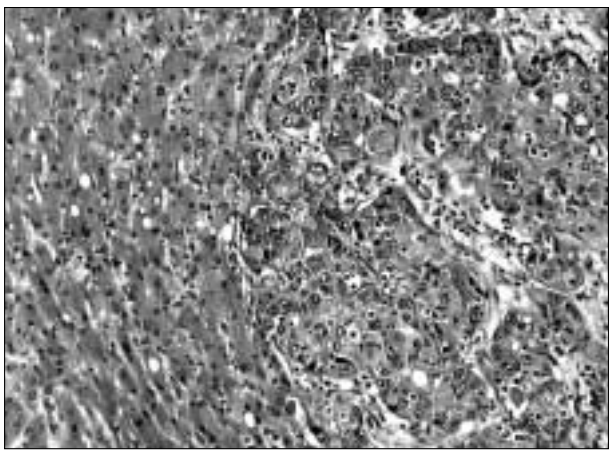

Figure 1
Microscopy of histology specimen: primary hepatocellular carcinoma

CONCLUSION

Tests for ANA are performed in an indirect immunofluorescence in cells derived from liver, kidney, possibly fibroblast or *in vitro* cultured nasopharynx carcinoma (Hep2). Most of these antibodies are directed against DNA- and/or RNA-protein complexes.[1,2] In rheumatic diseases ANA is often determined to obtain the probability of autoimmune diseases such as systemic lupus erythematosus (SLE), Sjögren's disease, scleroderma or polymyositis.[6] However low ANA titres may also be present in healthy persons: titres up to 1:80 occur in 13% of healthy individuals. This percentage of occurrence decreases to 3% for titres 1:320.[7,8] Similar to other autoantibodies ANA seropositivity is age dependent: 36% of the elderly (i.e. over 65 years) are ANA positive.[8]

Cytoplasmatic and nuclear autoantibodies may also occur in malignancy,[9,10] namely melanoma,[11] leukaemia,[12] lung carcinoma,[13] breast cancer,[14] gastrointestinal carcinoma,[15] as well as nasopharyngeal carcinoma.[16] In carcinomas 'single-stranded' DNA and histone proteins may behave as autoantigens. Most autoantigens in malignancy are

Table 1
Disorders associated with positivity for antinuclear antibodies (ANA)

RHEUMATIC DISORDERS	
SLE (anti-DNA)	
Systemic sclerosis	(anti-scl70)
Sjögren's disease	(SSA/SSB)
Mixed connective tissue disease	(anti-RNP)
Poly-/dermatomyositis	(anti-Jo1)
RA (RF)	

CAUSES IN GENERAL MEDICINE
Infections: Pfeiffer's disease, *E. coli*, malaria
Gastrointestinal disorders: IBD, PBC, liver cirrhosis, hepatocellular carcinoma
Pulmonary disorders: lung fibrosis, pulmonary hypertension
Endocrinopathy: Graves' disease
Haematology disorders: AML, ALL, CML, Hodgkin's disease
Neoplasia: melanoma, lung, breast and nasopharyngeal carcinoma

PHYSIOLOGICAL CAUSES
Pregnancy
Ageing
Drug-induced: procainamide, hydralazine

SLE = systemic lupus erythematosus, RA = rheumatoid arthritis, anti-DNA = anti-deoxynucleic acid, SSA = Sjögren syndrome antigen A, SSB = Sjögren syndrome antigen B, RF = rheumatoid factor, IBD = inflammatory bowel disease, PBC = primary biliary cirrhosis, AML = acute myeloid leukaemia, ALL = acute lymphatic leukaemia, CML = chronic lymphatic leukaemia.

Table 2
Tumour-associated autoantibodies possibly determined by in vitro ANA testing[7,8]

NUCLEIC PROTEINS	HEPATOCELLULAR CARCINOMA	LUNG CARCINOMA	BREAST CARCINOMA	OTHER
p53	+	+	-	Ovarian carcinoma
c-myc	-	+	+	SLE
c-myb	-	+	+	SLE
CENP-F	+	+	+	-
NOR-90	+	-	-	-
fibrillarin	+	-	-	-
B23	+	-	-	-

SLE = systemic lupus erythematosus.

still unknown, though during the last decade several nuclear antigenic epitopes have been recognised in primary hepatocellular carcinoma (HCC): fibrillarin and centromere protein p330[d]/CENP-F, both of which are involved in processing precursor ribosomal RNA, and the non-snRNP splicing factor SC35, which is involved in splicing messenger RNA.[7]

Another important potentially antigenic protein is p53. In normal cells p53 is responsible for temporarily arresting cell growth in response to certain types of molecular and/or biochemical damage. Several types of damage, physiological stress included, act by way of the p53 protein to trigger a programme of apoptosis, i.e. programmed cell suicide, to eliminate the damaged cell. Elimination of functioning p53 appears to be sufficient to inactivate the apoptotic machinery in many cancer cells. In HCC patients p53 is expressed supraphysiologically in 12 to 32%.[17,18] Characterisation of ANA specificities similar to these anti-p53-antibodies early in the course of tumours may help in understanding the processes involved in carcinogenesis. The immune response mechanism in carcinomas appears to have an antigen-driven character, in which abundant expression of antigenic target proteins plays a central role. In this process often mutant proteins with important cellular functions are involved.[19,20] In both primary hepatic carcinoma and breast cancer, tumour size and ANA seropositivity have been described to be inversely interrelated.[10,14] Patients with extensive disease are often cachectic and have a lower prevalence of ANA seropositivity.[10] In 204 HCC patients, Covini *et al.* found ANA positivity in 31% (speckled pattern in 15%, nucleolar in 10%, homogeous in 8%).[10]

The finding of a highly positive tumour-associated ANA, as in our patient, probably caused by p53 antibodies, is suggested in the literature to be a useful prognostic sign. As described earlier there is an association between idiopathic ANA and malignancy.[10] Six months after the primary hemihepatectomy the tumour had recurred and the patient was ANA positive, at a much lower titre. It is questionable whether a recurrent positivity of the ANA really suggests a poor prognosis, and this warrants further investigation.

In conclusion the presented case must not lead to over-estimation of the value of ANA.[21] Clinicians must bear in mind that a highly significant ANA which cannot be explained rheumatologically may well be evoked by arousal of autoimmunity due to abundant p53 expression in carcinoma, for example in primary hepatic carcinoma. Prompt analysis and specific treatment may be worthwhile, particularly when very high ANA titres are found.

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ANSWER TO PHOTO QUIZ (ON PAGE 290)

A WOMAN WITH A SWOLLEN, ERYTHEMATOUS AND TENDER THIGH

A subcutis biopsy showed a lobular panniculitis with signs of erythema nodosum. These eruptions may be associated with a wide variety of diseases. Extensive investigations excluded the presence of lymphomas, pancreatic abnormalities, sarcoidosis or enteropathies such as ulcerative colitis or Crohn's disease. Also no evidence was found for the presence of α-1 antitrypsin deficiency, complement deficiency, lupus erythematosus, leukaemia and infections including tuberculosis, *Streptococcus*, *Yersinia*, leptospirosis, histoplasmosis, Lyme borreliosis, hepatitis B and psittacosis.

DIAGNOSIS

We finally diagnosed Weber-Christian disease; a case of idiopathic lobular panniculitis associated with systemic involvement and no signs of an underlying disease. The patient was treated with a high dose of prednisone at 80 mg daily. She recovered very well within weeks, with full resolution of the skin eruptions and lowering of the erythrocyte sedimentation rate.

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'Portrait of Theo'

Bart Elfrink

This month's cover by Bart Elfrink shows a wood print on 185-gram Hahnemulle paper, entitled 'Portrait of Theo'. It is a portrait of his father, Theo Elfrink. A rhythmically gouged lino-cutting, it reveals an enormous sense of brightness and darkness, and great knowledge of portray.

Bart's prints can be described as modestly expressive. As descendant from a family of artists, he was raised in the art of observation. For several years now, his most important themes have been landscapes and portraits. Sometimes a combination of both; a portrait as landscape, a landscape of desire or a portrait of the landscape's soul.

A very limited edition (3) of the original print (size 40 x 40 cm) is available at Galerie Unita, Rijksstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands, e-mail: galerie-unita@planet.nl. Prices of these portraits by mutual agreement.

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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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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.

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