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The mission of the journal is to serve the need of the internist to practice up-to-date medicine and to keep track with important issues in health care. With this purpose we publish editorials, original articles, reviews, controversies, consensus reports, papers on specialty training and medical education, book reviews and correspondence.

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Editorial office ‘The Netherlands Journal of Medicine’
Geeralien Derksen-Willemsen
University Medical Centre St Radboud
Department of General Internal Medicine 541
PO Box 9101
6500 HB Nijmegen
The Netherlands
Tel.: +31 (0)24-361 04 59
Fax: +31 (0)24-354 17 34
E-mail: g.derksen@aig.umcn.nl

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Ehlers-Danlos syndrome

B.C.J. Hamel

Department of Human Genetics, Division of Clinical Genetics, University Medical Centre St Radboud, PO Box 9101, 6500 HB Nijmegen, the Netherlands, tel.: +31 (0)24-361 39 46, fax: +31 (0)24-356 50 26, e-mail: b.hamel@antrg.umcn.nl

In 2001, Schalkwijk et al. described, in eight patients from five families, a new autosomal recessive type of Ehlers-Danlos syndrome (EDS), caused by mutations in the tenascin-X (TNXB) gene and leading to a total deficiency of tenascin-X. Since then no other patients with tenascin-X deficiency have been published; apparently it is a rare type of EDS. This new type of EDS can be differentiated from the classic type, which it resembles most, by its mode of inheritance (autosomal recessive vs autosomal dominant) and the absence of abnormal scarring, which is one of the key features in the classic type of EDS. Remarkably, this new type is associated with congenital adrenal hyperplasia when it is due to a deletion encompassing the CYP21 gene.1,2

EDS consists of a group of inherited connective tissue disorders, mainly characterised by generalised joint hypermobility (Beighton hypermobility score of 5 or more), skin hyperextensibility (measured at the volar side of the underarm and/or at the flexed elbow), easy bruising and skin fragility (ranging from easy tearing after minor trauma to thin, broad scars and soft and velvety skin). It exhibits an enormous clinical and genetic heterogeneity. The monography by Brighten was the first comprehensive study of the syndrome and is still worth reading.3 In the latest classification six types are recognised (see table 1).4 EDS is not rare with an estimated prevalence of 1/5000. More than 90% of patients have either the classic or the hypermobility type.5

The classic type of EDS is characterised by all the main EDS features, namely hyperextensibility of the skin, broad and thin scarring (‘cigarette-paper’ scars), generalised joint hypermobility and easy bruising. Aortic dilatation and dissection is a rare complication of this EDS type. Well-known complications are recurrent (sub)luxations with chronic joint pain and early osteoarthrosis, hiatus hernia, anal

Table 1
Villefranche classification of Ehlers-Danlos syndrome

<table>
<thead>
<tr>
<th>NEW NAME</th>
<th>OLD NAME</th>
<th>INHERITANCE</th>
<th>MUTATED GENES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic type</td>
<td>Gravis = type I</td>
<td>AD</td>
<td>COL5A1, COL5A2</td>
</tr>
<tr>
<td></td>
<td>Mitis = type II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypermobility type</td>
<td>Hypermobile = type III</td>
<td>AD</td>
<td>?TNXB?</td>
</tr>
<tr>
<td>Vascular type</td>
<td>Arterial-echymotic = type IV</td>
<td>AD</td>
<td>COL3A1</td>
</tr>
<tr>
<td>Kyphoscoliosis type</td>
<td>Ocular-scoliotic = type VI</td>
<td>AR</td>
<td>Lysyl-hydroxylase</td>
</tr>
<tr>
<td>Arthrochalasia type</td>
<td>Arthrochalasis multiplex congenita = type VIIA and type VIIIB</td>
<td>AD</td>
<td>COL1A1, COL1A2</td>
</tr>
<tr>
<td>Dermatosparaxis type</td>
<td>Dermatosparaxis = type VIIIC</td>
<td>AR</td>
<td>Procollagen, N-peptidase</td>
</tr>
</tbody>
</table>

AD = autosomal dominant, AR = autosomal recessive.
prolapse, cervix uteri insufficiency with premature labour as a result and cicatrical hernia. There is a large interfamilial and intrafamilial clinical variability. The male-to-female ratio is almost equal to one. In about 50% of cases a mutation can be detected in cultured fibroblasts in the COL5A1 or COL5A2 genes.

The hypermobility type of EDS has generalised joint hypermobility and mildly hyperextensible and/or soft and velvety skin as its main features. The main problem is the joint hypermobility with its sequelae, recurrent (sub)luxations of mainly shoulder, patella and jaw, chronic joint pain which can be severe and invalidating, and early arthrosis. Complications related to internal organs are extremely rare. It should be distinguished from the benign joint hypermobility syndrome (BJHS), which lacks the skin symptoms of EDS. However, there is much debate whether BJHS and the hypermobility type of EDS are not one and the same disorder.\(^6\) Personally, I adhere to the strict definition of BJHS, namely generalised joint hypermobility with Beighton scores \(\geq 5\), joint pain during a longer period in several joints and absence of skin features. Recently, Zweers et al. reported their tenascin-X findings in two groups of patients.\(^7\) First, the haploinsufficient family members \((n=20,\) mainly siblings, children, parents) of their patients with absent tenasin-X due to homozygous or compound heterozygous \(TNXB\) mutations: in this group, nine out of 14 women – and none of the six males – showed generalised joint hypermobility and four of these nine women also had a velvety skin. The second group consisted of 80 hypermobility type EDS patients, diagnosed by a medical specialist and recruited through the Dutch EDS patient organisation. In six \((7.5\%;\) all females) of these, tenasin-X haploinsufficiency was detected. Four of these six patients were examined: two had Beighton scores \(<5\) and no abnormal skin, while of the two with Beighton scores \(\geq 5\), one had normal skin. What does this tell us? Firstly, that probably quite a few of the 80 hypermobility-type EDS patients do not have the hypermobility type of EDS nor the strictly defined BJHS, secondly, that a better controlled study is needed to clarify the role of tenasin-X in hypermobility disorders, and thirdly that most likely skin extensibility and joint mobility is the end result of the involvement of many genes and exogeneous factors (‘multifactorial’). This is illustrated in among other ways by the fact that haploinsufficiency for tenasin-X does exist without phenotype, for a presumed autosomal dominant disorder far too many patients are sporadic (= not familial) and almost all patients with the hypermobility type of EDS and BJHS are female.

The vascular type of EDS is more rare than the two previous ones but it is the most serious and life-threatening of all. Thin skin due to lack of subcutaneous fat with prominent venous pattern and a peculiar face and easy bruising are hallmarks as well the easy rupturing of internal organs as the large arteries, large intestines and uterus. Pregnant women with the vascular type of EDS are at an increased risk for severe complications. It is caused by mutations in \(COL3A1\) gene (cultured fibroblasts), which are detectable in nearly all patients.

The other three types are even rarer and will not be discussed in detail. Apart from these six EDS types and the new tenasin-X deficient type, there are more poorly defined EDS types and single family ‘types’.\(^4\) From clinical practice it is known that not every patient with an EDS phenotype can be classified in one of the well-defined types.

EDS is diagnosed and classified on the basis of history, family history and physical examination with laboratory confirmation when possible and indicated, at the protein level (tenasin-X in serum, collagen 1, 3 and 5 in fibroblasts) and/or at the molecular level (see text and table 1). Interestingly, some often heard complaints are not readily found in textbooks on EDS: easy fatigue is one of these as is the ineffectiveness of local anaesthesia; the former is particularly clinically important and often distressing. Single reports on EDS patients with various clotting abnormalities (for example von Willebrand disease, factor IX deficiency, factor XIII deficiency, platelet release defect, fibronectin deficiency) have been published, but the cause of the easy bruising is much more likely to be due to vessel wall fragility than to a coagulation defect.\(^5\)

Management of EDS is largely supportive (e.g. physiotherapy, ergotherapy, rehabilitation) and preventive (e.g. surgical precautions, avoiding certain professions and sports, avoiding overweight); it is rarely surgical (e.g. arthrodesis). Genetic counselling is part of the management.

Differential diagnosis of EDS includes other connective tissue disorders such as Marfan’s syndrome, cutis laxa and osteogenesis imperfecta, but also a host of other diagnoses depending on the presenting complaint/symptom. Since it is also of great importance for the internist to recognise EDS in order to start timely management and to avoid unnecessary investigations, it is therefore wise of the editors of the Netherlands Journal of Medicine to publish the paper by Peeters et al.,\(^8\) not so much to draw attention to this rare type of EDS, but more to EDS in general.

**NOTE**

There is an active Dutch patient organisation (Vereniging van Ehlers-Danlos patienten, www.ehlers-danlos.nl). In the Netherlands, the molecular and protein diagnostics of EDS (for types see table 1) are performed at the VU University Medical Center, Department of Clinical and Human Genetics, Laboratory for DNA and Protein Diagnostics, Amsterdam, head Dr G.Pals.
REFERENCES


... Tenascin-X?
I thought you meant that new chewing gum!
Hypothesis: normalisation of cytokine dysbalance explains the favourable effects of strict glucose regulation in the critically ill

P. Pickkers1,2*, A. Hoedemaekers1,3, M.G. Netea3, B.E. de Galan3, P. Smits2, J.G. van der Hoeven1, M. van Deuren3

Departments of 1Intensive Care Medicine (519), 2Pharmacology-Toxicology and 3Internal Medicine, University Medical Centre St Radboud, Nijmegen, the Netherlands, tel.: +31 (0)24-361 74 49, e-mail: p.pickkers@ic.umcn.nl, * corresponding author

ABSTRACT

Recent trials investigating the effects of strict glucose regulation in critically ill patients have shown impressive reductions in morbidity and mortality. Although the literature focuses on the possible toxic effects of high blood glucose levels, the underlying mechanism for this improvement is unclear. We hypothesise that strict glucose regulation results in modulation of cytokine production, leading to a shift towards a more anti-inflammatory pattern. This shift in the cytokine balance accounts for the reduction in morbidity and mortality.

To support our hypothesis, effects of glucose and insulin on cytokine release and effects of glucose, insulin, and cytokines on host defence, cardiac function and coagulation will be reviewed.

INTRODUCTION

Diabetic patients are at risk for cardiovascular and various infectious diseases while infections will often run a complicated course. The reason for this increased susceptibility to infections may be an impaired immune defence, microvascular and macrovascular comorbidity, the relative high number of medical interventions or – viewed from the microbial site – an altered adherence of micro-organisms to human cells under hyperglycaemic conditions. Reportedly, postoperative hyperglycaemia is an independent risk factor for the development of infectious complications. A large recent study by Van den Berghe et al. in critically ill patients showed that maintenance of blood glucose levels between 4.4 and 6.1 mmol/l results in a 42% reduction in mortality compared with conventional treatment aimed at blood glucose levels between 9.9 and 11.1 mmol/l. Patients with multiple-organ failure and a proven septic focus demonstrated the greatest improvement with intensive insulin therapy. In addition, in patients with acute myocardial infarction maintenance of blood glucose levels below 11.9 mmol/l has been shown to increase the success rate of thrombolysis, preserves myocardial function, and improves long-term outcome. How the strict control of blood glucose reduces morbidity and mortality is not known, but it is thought that the mechanism may be related either to a direct effect of normalisation of hyperglycaemia or to the concomitant higher insulin levels.

There is a striking similarity between the effects of high glucose levels on host defence, cardiac function and coagulation and the observation of elevated systemic levels of proinflammatory cytokines during critical illness. Cytokines, low-molecular-weight proteins produced by various immune-competent cells, have important modulating effects on the immune response. Cytokines have autocrine, paracrine and endocrine effects. Whereas the local autocrine and paracrine effects may be beneficial in...
It has been shown that insulin deficiency or from long-term effects from short-term metabolic effects of hyperglycaemia and overproduction of free radicals. These defects may result in decreased phagocytosis, decreased bacterial killing and decreased chemotaxis, increased adherence to endothelium, in neutrophil function have been observed such as decreased rate of glycolysis and an increased glucose consumption after the addition of small amounts of insulin. In diabetic patients, a variety of abnormalities in neutrophil function have been observed such as decreased chemotaxis, increased adherence to endothelium, decreased phagocytosis, decreased bacterial killing and overproduction of free radicals. These defects may result from short-term metabolic effects of hyperglycaemia and (relative) insulin deficiency or from long-term effects such as increased glycation.

It has been shown in vitro that D-glucose and insulin, alone or in combination, improve chemotaxis of neutrophils from healthy humans. D-glucose alone is able to increase neutrophil locomotion in a dose-dependent fashion. However, as studied in vivo, N-formyl-methionyl-leucyl-phenylalanine (fMLP) and complement-induced chemotaxis is significantly lower in diabetic patients than in healthy controls, probably because of the increased expression of the adhesion promoting β2-integrins CD11b and CD11c. Because in addition, glucose increases the expression in vitro of adhesion molecules on endothelium such as E-selectin, intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), hyperglycaemia will promote the binding of leukocytes to endothelium. Recently, it was demonstrated that K\textsubscript{ATP} channel blockade with glibenclamide inhibited fMLP and lipopolysaccharide-induced neutrophil activation and migration in vitro and in vivo, suggesting that, independent of glucose and insulin, treatment of type 2 diabetic patients with glibenclamide could be detrimental to immune function. In type 2 diabetic patients circulating levels of cytokines and adhesion molecules are increased following a meal rich in carbohydrates. This effect was inhibited by pretreatment with high doses of vitamin C and E, indicating that an oxidative mechanism mediates this effect.

Phagocytosis is impaired in diabetes. This impairment is at least partly mediated by elevated levels of cytosolic calcium. In a prospective cohort study of patients with type 2 diabetes, higher fasting glucose levels were correlated with higher cytosolic calcium levels in neutrophils and with decreased phagocytic activity. In type 2 diabetes mellitus this impaired phagocytic activity can be restored by glibenclamide therapy, either as a result of the glibenclamide-stimulated insulin production or decreased serum glucose levels. In diabetes, ingested bacteria seem to be protected from killing by intracellular bactericidal mechanisms. Under hyperglycaemic conditions, proteins may be glycated resulting in the formation of so-called advanced glycation end products (AGEs). Recent studies have shown that these AGEs can bind to the corresponding receptor on neutrophils and impair intracellular bacterial killing. Delamaire et al. reported that, although elevated in the unstimulated state, bactericidal activity of stimulated neutrophils was significantly depressed in diabetic patients. Neutrophils from diabetic patients show increased levels of basal free radical release as measured by chemoluminescence. This increased free radical production, in combination with increased adhesion, may induce local lesions in the vascular endothelium. Although all these defects in leucocyte function have been reported in diabetic patients, the exact mechanism and the effects of short-term glucose control are still not clear. We speculate that high glucose concentrations influence host defence by the modulation of cytokine synthesis.

**THE HYPOTHESIS**

Normalisation of hyperglycaemia in acute and critically ill patients will shift the cytokine profile from a systemic proinflammatory status to a more balanced anti-inflammatory condition. By this mechanism, normoglycaemia improves and restores host defence, haemodynamics and coagulation abnormalities and decreases morbidity and mortality.

**EFFECTS OF GLUCOSE AND INSULIN ON HOST DEFENCE, ROLE OF CYTOKINES**

While glucose is freely permeable across the cell membrane of leucocytes in diabetic patients, these cells display a decreased rate of glycolysis and an increased glucose consumption after the addition of small amounts of insulin. In diabetic patients, a variety of abnormalities in neutrophil function have been observed such as decreased chemotaxis, increased adherence to endothelium, decreased phagocytosis, decreased bacterial killing and overproduction of free radicals. These defects may result from short-term metabolic effects of hyperglycaemia and (relative) insulin deficiency or from long-term effects such as increased glycation.

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This mechanism is sustained by a recent report by Morohoshi et al. that showed a glucose-dependent increase in basal TNFα and IL-6 production in human monocytes in vitro. Similarly, studies by our group demonstrated a glucose-dependent increased production of TNFα by peripheral blood cells in vitro after stimulation with lipopolysaccharide, whereas glucose does not influence the production of the anti-inflammatory cytokine IL-10. We also demonstrated that in vivo induced hyperglycaemia in hypoglycaemic human clamp models (achieved glucose levels 5.0, 3.5, and 2.5 mmol/l) resulted in a down-modulation of LPS-induced TNFα synthesis. Together, these studies strongly suggest that glucose may stimulate the production of proinflammatory cytokines such as TNFα and IL-6, with no effect on the anti-inflammatory cytokine IL-10. Therefore, normalisation of hyperglycaemia may result in a shift of cytokine production towards a more balanced anti-inflammatory profile. In addition to the effect of glucose on the production of pro- and anti-inflammatory cytokines, several effects of insulin on cytokine production have been shown. Satomi et al. demonstrated that insulin blocks TNFα production by peritoneal macrophages. Insulin has been shown to have a potential anti-inflammatory activity, since it inhibits the expression of the proinflammatory adhesion molecule ICAM-1 by endothelial cells and increases the expression of the mRNA for macrophage migration inhibitory factor. Rats treated with recombinant TNFα have a significant decrease in food intake, nitrogen balance and body weight compared with saline-treated control rats. Concurrent insulin administration reversed all these changes. Five days of TNFα treatment caused severe interstitial pneumonitis, periportal hepatitis and increased wet organ weight of heart, lung, kidney and spleen. Concurrent insulin treatment led to near total reversal of these pathological changes. However, as systemic insulin administration results in lower glucose concentrations, the observed effects cannot be attributed to insulin per se. Of the several questions that can be addressed, perhaps the most intriguing is how glucose mediates the TNFα production. A possible explanation involves nuclear factor κB (NFκB). The inducible transcription factor NFκB was discovered in and is present in the cytoplasm of many different cell types, such as leucocytes, endothelial and epithelial cells. Activation of this protein initiates its translocation into the nucleus where it binds to specific sequences in the promoter regions of target genes. This will result in an increased rate of transcription of the gene, more messenger RNA (mRNA) and more protein. NFκB plays a pivotal role in the regulation of the synthesis of various proinflammatory proteins, including cytokines, cell-adhesion molecules and inducible nitric oxide synthase. In sepsis, NFκB expression is upregulated and a persistently increased expression correlates with nonsurvival. The production of NFκB is stimulated by lipopolysaccharide (LPS), TNFα, IL-1, and phorbol 12-myristate 13-acetate. The anti-inflammatory cytokine IL-10 inhibits NFκB formation. In type 2 diabetes it has been shown that IL-1β inhibits pancreatic β cell function and promotes Fas-triggered apoptosis in part by activating NFκB. This suggests that an inflammatory process is involved in the pathogenesis of glucotoxicity in type 2 diabetes, which is mediated by NFκB. In Gram-negative sepsis, LPS or endotoxin binds to several receptors of which CD14 is one of the most extensively studied. After binding to LPS, this receptor forms a complex with the toll-like receptor 4 (TLR4). Activation of TLR4 results in the activation of the NFκB signalling pathway and subsequently to the production of cytokines such as TNFα, IL-1, IL-2, IL-6, IL-8, and IL-12, and interferon-β (IFNβ). TNFα, in a positive regulatory loop, can lead to further excessive activation of NFκB and to cytokine-mediated effects such as endothelial cell injury, disseminated intravascular coagulation, septic shock or death. Hyperglycaemia rapidly induces activation of NFκB within hours of its onset. NFκB activation induced by elevated concentrations of carbohydrates is associated with increased monocyte adhesion to endothelial cells and the generation of oxygen radicals. Adherence of leucocytes to human umbilical vein endothelial cells was strongly augmented by elevation of the glucose concentration in the perfusate from 5 mmol/l to 30 mmol/l. In the same experiment it was shown that this effect was paralleled by an intense NFκB activation. So, direct effects of glucose on NFκB activation may well be the initiator of the deleterious effects of increased systemic cytokine production. However, as NFκB is also involved in the stimulation of some anti-inflammatory cytokines, more subtle stimulation of intracellular pathways is likely to be involved. Oxidative stress-mediated cellular damage may be involved in hyperglycaemia-induced activation (reviewed in), as was shown by treating diabetic patients with antioxidants that significantly suppressed NFκB activation. By inhibiting NFκB activation, transcription of genes under NFκB control, such as adhesion molecules and cytokines, is prevented. Macrophage migration inhibitory factor (MIF) is a pro-inflammatory chemokine produced primarily by the anterior pituitary gland. LPS-induced secretion of MIF from the pituitary gland and from circulating/tissue resident monocyte/macrophages is thought to play an important role in the pathogenesis of endotoxaemia and sepsis and might be an integral part of the host’s systemic stress response. In mice that received endotoxin, there was a dramatic fall in the pituitary content of MIF, associated with a concomitant increase in plasma levels of MIF. In vivo studies in rats have shown that MIF protein is released from the pituitary, adrenal gland, liver, spleen, lung, kidney and skin within six hours of LPS injection. Upon release, MIF is directly proinflammatory by activating...
or promoting cytokine expression (TNFα, IL-1β, IL-2, IL-6, IL-8, and interferon-γ). From this data it is stated that 'MIF’s position within the cytokine cascade is to act in concert with glucocorticoids to control the set point of the immune and inflammatory response'.

Similarly to TNFα, high MIF levels are closely linked with poor outcome in patients with systemic inflammatory response syndrome (SIRS). Again, there is a link with glucose metabolism: the insulin-secreting β cells in the pancreas express MIF, and both MIF and insulin colocalise by immuno-cytochemistry within the secretory granules. MIF is secreted in response to glucose stimulation in a time- and concentration-dependent fashion. In turn, exogenously administered MIF potentiates insulin release. MIF thus plays an important regulatory role in carbohydrate metabolism and its secretion is regulated by glucose. Through effects on MIF, strict glucose regulation could influence the pro-/anti-inflammatory cytokine balance.

**EFFECTS OF GLUCOSE AND INSULIN ON CARDIAC FUNCTION, ROLE OF CYTOKINES**

Inflammation plays a pathogenic role in the development of acute coronary syndromes. Increased levels of TNFα, IL-1β, IL-6, IL-8 and MIF have been observed after acute myocardial infarction and postinfarction IL-6 levels correlate significantly with the extent of myocardial tissue damage. TNFα production forms part of an important intrinsic myocardial stress response system to injury. Within the myocardium TNFα is not only confined to the infarct and peri-infarct zone, but is also expressed in cardiac myocytes of the contralateral myocardium. As can be concluded from a study in mice, TNFα has, apart from its pathogenic role in the acute inflammatory response during the acute ischaemia, also a protective role in limiting the ischaemia-induced apoptosis. Several studies have suggested that treatment of acute myocardial infarction with infusions of glucose, insulin and potassium is beneficial. It remains unclear whether normalisation of hyperglycaemia or the higher insulin levels account for these beneficial effects. Insulin has been shown to attenuate infarct size in the isolated rat heart, independent of the presence of glucose, and it was also demonstrated that infarct size was linearly related to blood glucose concentration during acute hyperglycaemia independent of insulin concentration in dogs. The DIGAMI study was initiated to test the hypothesis that rapid improvement of metabolic control in diabetic patients with acute myocardial infarction improves short- and long-term outcome. Patients were randomised to insulin-glucose infusion followed by multidose subcutaneous insulin therapy or conventional therapy. In the intensive therapy group mortality rate was reduced by 30% after one year. This effect is most likely the result of better local metabolic control in the myocardium or, in accordance to the presently presented hypothesis, to an altered cytokine-mediated inflammatory response.

**EFFECTS OF GLUCOSE AND INSULIN ON COAGULATION, ROLE OF CYTOKINES**

In critically ill patients, derangement of the coagulation system comprises enhanced activation of coagulation, depression of inhibitory mechanisms of coagulation, and inhibition of fibrinolysis. An antifibrinolytic endothelium phenotype is associated with multorgan failure and mortality. Derangement of the coagulation system appears to be mediated by various cytokines and may contribute to the pathogenesis of multiple organ failure. In human umbilical vein endothelial cells, TNFα alters the coagulant activity by stimulation of the production and surface expression of tissue factor and by inhibition

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**Figure 1**

Representation of the hypothesis. Correction of high glucose concentration results in less NFκB activation and down-modulation of TNFα production, resulting in less spill-over to the systemic circulation. The production of the anti-inflammatory cytokine IL-10 is not influenced by glucose. This leads to a new pro- and anti-inflammatory cytokine balance with favourable effects on host defence, myocardial function, and coagulation. In the presence of little experimental evidence, the possible role of insulin remains questionable.
of anticoagulant mechanism, particularly the activation of the protein C system.\(^6\)\(^7\)\(^8\) In healthy human volunteers, infusion of TNFα activates the coagulation system identically to what is seen in septic patients or in volunteers after endotoxin infusion,\(^6\)\(^9\)\(^70\) and this activation of the coagulation seems to be, at least initially, dependent on activation of the extrinsic pathway of blood coagulation.\(^71\) In septic patients, the fibrinolytic system becomes initially activated and subsequently inhibited.\(^72\) Blockade of TNFα with monoclonal antibodies inhibits the fibrinolytic response upon the administration of endotoxin.\(^73\)\(^74\) The mechanisms underlying the effects of TNFα on the fibrinolytic system are not completely clear. It is suggested that the rapid effects are not due to enhanced gene expression observed \(\textit{in vitro},\)\(^75\)\(^76\)\(^77\) but to release of stored plasminogen activators probably from the vascular endothelium and endothelin-activated platelets.

The level of plasminogen activator inhibitor type I (PAI), an inhibitor of fibrinolysis, is higher in diabetic patients as compared with nondiabetics, and associated with the occurrence of myocardial infarction.\(^78\) Systemic insulin treatment decreases the plasma activity of PAI in type 2 diabetic patients.\(^79\) Human administration of recombinant IL-6, another proinflammatory cytokine, resulted in an increased thrombin generation\(^80\) and antibodies directed against IL-6 are able to completely abrogate the endotoxin-induced activation of coagulation in chimpanzees.\(^81\) Also, diabetes has been associated with platelet hyperactivity to agonists \(\textit{in vitro},\) and alterations in a number of mechanisms involved in platelet activation occur in diabetic platelets. These alterations include increased presence of glycoprotein receptors for agonists and adhesive proteins on the platelet surface, increased fibrinogen binding, decreased membrane fluidity, enhanced arachidonate pathway activation with increased thromboxane A\(_2\) formation, and increased phosphoinositide turnover leading to increased inositol triphosphate production, calcium mobilisation, and protein phosphorylation.\(^82\) Tight glycaemic control with insulin therapy reduces the excretion levels of a thromboxane metabolite by approximately \(50\%\).\(^83\) Again, the mechanism responsible for this effect is unclear.\(^84\) In accordance with our hypothesis, the effect of systemic insulin treatment could be explained by better glucose control, subsequent inhibition of proinflammatory cytokines, resulting in a reduced cytokine-induced procoagulatory state.

Recently, it was shown that suppletion of the anticoagulatory protein recombinant activated protein C (aPC) reduces the mortality in critically ill patients.\(^85\) Apart from the insulin study by Van de Berghe et al.,\(^4\) this is the only immune-modulatory strategy so far that has proven to be efficacious. The effects of aPC, however, appear to involve more than its antithrombotic activity. Similar to one of the effects of strict glucose control, aPC influences translocation of NFκB and cytokine production in an anti-inflammatory way.\(^86\) Normalisation of hyperglycaemia may therefore be beneficial either directly or by a restoration of the cytokine balance, through a positive effect on the immune function, through the improvement of haemodynamics or through a decrease of the undesirable procoagulatory state.

### Table 1

**Summary of the effects of hyperglycaemia (compared with normoglycaemia)**

<table>
<thead>
<tr>
<th>EFFECTS ON HOST DEFENCE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NFκB induction</td>
<td>↑</td>
</tr>
<tr>
<td>Production of proinflammatory cytokines, MIF</td>
<td>↑</td>
</tr>
<tr>
<td>Production of anti-inflammatory cytokines</td>
<td>=</td>
</tr>
<tr>
<td>Expression of adhesion molecules</td>
<td>↑</td>
</tr>
<tr>
<td>Binding of leucocytes to endothelium</td>
<td>↓</td>
</tr>
<tr>
<td>Phagocytic activation</td>
<td>↑</td>
</tr>
<tr>
<td>Intracellular bacterial killing</td>
<td>↑</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EFFECTS ON CARDIAC FUNCTION</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Infarct size</td>
<td>↑</td>
</tr>
<tr>
<td>Postinfarction mortality</td>
<td>↑</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EFFECTS ON COAGULATION</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PA-1 concentration</td>
<td>↑</td>
</tr>
<tr>
<td>Thromboxane concentration</td>
<td>↑</td>
</tr>
<tr>
<td>Platelet activity</td>
<td>↑</td>
</tr>
</tbody>
</table>

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**Conclusion and Future Directions**

Strict control of plasma glucose in acute and critically ill diabetic and nondiabetic patients has been shown to improve outcome in several clinical settings. The mechanism of action of these beneficial effects is not known, although in critically ill patients, the control of glucose levels rather than the absolute levels of insulin appear to account for the positive effects of strict regulation.\(^86\) From the literature and our own studies it can be concluded that hyperglycaemia augments the production of proinflammatory cytokines. There is a striking similarity between the effects of glucose on host defence, cardiac performance and coagulation and the proinflammatory cytokine status associated with critical illness. Although in acute or critically ill patients various mutually dependent overlapping and redundant pathophysiological mechanisms are known to influence the clinical course in a complex fashion, we claim that the modulation of cytokines explains the beneficial effect of a more strict glycaemic control. Cognisant the...
beneficial local autocrine and paracrine action and the potentially deleterious systemic endocrine effect of pro-inflammatory cytokines, we suggest that future studies to test this hypothesis encompass local and systemic cytokine production. Further understanding of the mechanism by which intensive therapy with insulin reduces morbidity and mortality in acute and critically ill patients will probably take place at the crossroads of metabolic control, immune response, cardiac performance and coagulation.

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33. Sen R, Baltimore D. Multiple nuclear factors interact with the 


ABSTRACT
The arterial baroreflex buffers abrupt transients of blood pressure and prevents pressure from rising or falling excessively. In experimental animals, baroreceptor denervation results in temporary or permanent increases in blood pressure level and variability, depending on the extent of denervation. In humans, the clinical syndrome of baroreflex failure may arise from denervation of carotid baroreceptors following carotid body tumour resection, carotid artery surgery, neck irradiation and neck trauma. The syndrome is characterised by acute malignant hypertension and tachycardia followed by labile hypertension and hypotension. Baroreflex failure can be a cause of hypertension and should also be considered in the differential diagnosis of pheochromocytoma. Patients with suspected baroreflex failure should be referred to specialised centres for diagnostic testing and treatment.

BARORECEPTORS
In the late 1920s, Hering and Koch were the first to recognise the reflex nature of changes in heart rate and blood pressure evoked by external massage of the neck. The afferents were tracked as nerve endings at the carotid bifurcation. The arterial baroreflex buffers abrupt transients of blood pressure and originates from stretch sensitive receptors in the arterial wall of the carotid sinus and the aortic arch and large vessels of the thorax. Afferent fibres from carotid sinus baroreceptors join the glossopharyngeal nerve (ninth cranial nerve) and project to the nucleus tractus solitarii in the dorsal medulla, which in turn projects to efferent cardiovascular neurones in the medulla. In addition to carotid baroreceptors, stretch-sensitive baroreceptors are also located in the aortic arch, heart and large pulmonary vessels. The extra-carotid baroreceptors transmit their afferent information along with the vagal nerves to the same brain stem nuclei. The efferent limbs of the baroreflex loop consist of sympathetic and parasympathetic fibres to the heart as well as to blood vessels.

EXPERIMENTAL AND IATROGENIC DENERVATION OF BARORECEPTORS
Arterial baroreceptors provide a tonic inhibitory influence on sympathetic tone, thus controlling peripheral vasoconstriction and cardiac output. Therefore, baroreceptor denervation would be expected to result in a sustained increase in sympathetic tone and, as a consequence, a sustained increase in blood pressure. Indeed, experimental denervation of carotid, aortic and cardiopulmonary baroreceptors in dogs produces a persistent increase in blood pressure level and variability. Following selective carotid and/or aortic baroreceptor denervation, the increase in blood pressure and heart rate is usually temporary. The first report on baroreceptor denervation in humans appeared in the 1930s. Unilateral section of the glossopharyngeal nerve in five patients with glossopharyngeal neuralgia produced a prompt and pronounced rise in blood pressure, which lasted 5 to 12 days. In 1956, a patient died from a fatal hypertensive crisis following unilateral carotid sinus denervation, which had been performed for the relief of recurrent syncope due to a
hypersensitive carotid sinus syndrome. In 1985, Fagius and Wallin reported the effects of experimental chemical blockade of carotid baroreceptors in humans. These authors performed bilateral anaesthetic blockade of vagal and glossopharyngeal nerves upon each other, which resulted in an elevation of blood pressure and tachycardia, accompanied by a strong increase in muscle sympathetic nerve activity.

Apart from these experimental studies, inadvertent baroreceptor denervation may occur as a complication of bilateral carotid body tumour resection, radiotherapy and surgery for laryngeal/pharyngeal carcinoma, bilateral and unilateral carotid endarterectomy and trauma of the neck. Disruption of the baroreflex has also been reported in the event of ischaemic or neurodegenerative lesion of the nucleus tractus solitarii. The clinical syndrome of baroreflex failure has now been characterised as a separate clinical entity.

**BAROREFLEX FAILURE SYNDROME**

The acute form of baroreflex failure is encountered following loss of glossopharyngeal or carotid sinus nerve function due to surgical intervention or accidental injury. It is characterised by severe, unremitting hypertension, tachycardia, and headache (table 1). The systolic blood pressure typically exceeds 250 mmHg, which may lead to hypertensive encephalopathy and (fatal) cerebral haemorrhage. Hypertensive crisis may evolve over days and weeks into the more chronic volatile hypertension phase. In addition, volatile hypertension may result from a gradual decline in baroreflex function due to neck irradiation. Irradiation may affect the stretch-induced afferent baroreceptor activity due to direct trauma of baroreceptors or by inducing atherosclerosis and fibrosis of the carotid sinus arterial wall. Volatile hypertension due to baroreflex failure is characterised by paroxysms of abrupt sympathetic activation, including excessive increments in plasma catecholamine levels. Surges of blood pressure and tachycardia may occur spontaneously or are elicited by mental stress or physical stimuli such as exercise, cold and sexual arousal. These bouts of sympathetic activation may be accompanied by severe headache, palpitations, diaphoresis, light-headedness and anxiety. Hypertensive pressure may be increased. In addition, emotional instability appears to be a prominent feature in this phase of baroreflex failure. Apart from hypertensive surges, hypotensive valleys may occur during sleep. In rare cases, inadequate baroreflex buffering of cardiovascular effereence is the most prominent feature, resulting in malignant vagotonia with hypotension, bradycardia, and asystole.

Accompanying symptoms of this so-called ‘selective baroreflex failure’ include fatigue and dizziness, with possible progression to frank syncope.

**WHEN SHOULD BAROREFLEX FAILURE BE CONSIDERED?**

A history of prior (iatrogenic) trauma of the neck is the most important clue in suspecting the diagnosis of baroreflex failure. The diagnosis of baroreflex failure should be considered in patients with a negative work-up for pheochromocytoma, since this carries a strong clinical resemblance to baroreflex failure. Apart from pheochromocytoma, which should be ruled out by the proper biochemical and radiographic investigations, the differential diagnosis of baroreflex failure includes paroxysmal tachycardia, migraine, hyperthyroidism, renovascular hypertension, alcohol withdrawal, drug use (e.g. amphetamines or cocaine), mastocytosis, carcinoid syndrome, intracranial lesions and psychological disorders (panic attack, generalised anxiety disorder). Labile hypertension and hypotension can be demonstrated by a 24-hour ambulatory blood pressure recording. Patients with a suspicion of baroreflex failure should be referred for further investigations.

### Table 1

**Symptoms and signs of baroreflex failure**

<table>
<thead>
<tr>
<th>Involvement</th>
<th>Symptoms and signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute phase</td>
<td>Severe sustained elevation of blood pressure (systolic pressure typically &gt;250 mmHg)</td>
</tr>
<tr>
<td></td>
<td>Tachycardia</td>
</tr>
<tr>
<td></td>
<td>Elevation of plasma catecholamines</td>
</tr>
<tr>
<td></td>
<td>Headache</td>
</tr>
<tr>
<td></td>
<td>Complications of hypertension</td>
</tr>
<tr>
<td></td>
<td>Encephalopathy</td>
</tr>
<tr>
<td></td>
<td>Cerebral haemorrhage</td>
</tr>
<tr>
<td>Chronic phase</td>
<td>Common</td>
</tr>
<tr>
<td></td>
<td>Volatile hypertension and tachycardia</td>
</tr>
<tr>
<td></td>
<td>Palpitations</td>
</tr>
<tr>
<td></td>
<td>Headache</td>
</tr>
<tr>
<td></td>
<td>Diaphoresis</td>
</tr>
<tr>
<td></td>
<td>Light headedness</td>
</tr>
<tr>
<td></td>
<td>Anxiety</td>
</tr>
<tr>
<td></td>
<td>Emotional instability</td>
</tr>
<tr>
<td></td>
<td>Increased intraocular pressure</td>
</tr>
<tr>
<td></td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>Hypotension (during sleep)</td>
</tr>
<tr>
<td></td>
<td>Bradycardia, asystole</td>
</tr>
<tr>
<td></td>
<td>Fatigue</td>
</tr>
<tr>
<td></td>
<td>(Orthostatic) dizziness</td>
</tr>
<tr>
<td></td>
<td>Syncope</td>
</tr>
</tbody>
</table>
to a specialised centre for the evaluation of autonomic cardiovascular function. Disruption of the baroreflex arch is demonstrated by absence of reflex bradycardia and tachycardia in response to intravenous injection of pressor drugs such as phenylephrine and depressor drugs as nitroprusside, respectively.12,31 The baroreflex modulation of muscle sympathetic nerve activity can be assessed by micro-neurography of sympathetic fibres within the peroneal nerve (figure 1).32,33 Additional cardiovascular reflex tests such as Valsalva’s manoeuvre, standing up, forced breathing, cold face test, cold pressor test and mental arithmetic34 can be used to tease out the localisation of the baroreflex lesion.34 Baroreflex failure is essentially different from autonomic neuropathy, which is either primary (pure autonomic failure, multiple system atrophy) or secondary (e.g. diabetes mellitus). In contrast to baroreflex failure which is caused by lesions of the afferent innervation of baroreceptors, autonomic neuropathy is characterised by abnormal efferent innervation to the heart and resistance vessels. The key feature of autonomic neuropathy is (severe) orthostatic hypotension.35 Syncope due to the hypersensitive carotid sinus syndrome is caused by excessive afferent nerve impulses towards the brainstem, causing cardioinhibition and/or vasodepression resulting in syncope.16

**TREATMENT AND PROGNOSIS**

Information on the treatment of acute, postsurgical baroreflex failure is scarce and relies on observational case studies. As in any form of hypertensive crisis, antihypertensive treatment in baroreflex failure is aimed at the prevention of hypertensive encephalopathy, (cerebral) haemorrhage, myocardial infarction, heart failure and hypertensive retinopathy. Haemodynamic monitoring of these patients in a medium or intensive care unit is warranted in the acute phase. Theoretically, intravenous administration of drugs with a short half-life is preferred in view of the strong blood pressure lability. Drugs that have been used in this setting include nitroprusside, phentolamine and labetalol.37,38 Apart from antihypertensive treatment, adequate analgesic and sedative therapy for relief of postsurgical discomfort and baroreflex failure related symptoms such as headache and palpitations are indicated.

In the phase of labile hypertension, the primary goal of therapy is to reduce the frequency and magnitude of surges in blood pressure and heart rate. Clonidine, a centrally and peripherally acting α-adrenoreceptor and imidazoline

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**Figure 1**

Assessment of baroreflex control of heart rate (upper panel) and muscle sympathetic nerve activity (lower panel) in a normal subject. Nitroprusside-induced hypotension elicits a baroreflex mediated increase in heart rate and muscle sympathetic nerve activity. BP = blood pressure, HR = heart rate.
agonist, has been shown to reduce both frequency and severity of pressor surges. Both central inhibition of noradrenergic neurotransmission and sedative effects may contribute to the beneficial effect of clonidine in baroreflex failure. If tolerated by the patient, daily doses as high as 1.2 to 2.4 mg may be required. The α1-adrenoceptor blocker phenoxybenzamine has also been shown to reduce the magnitude of blood pressure surges. In patients who have been well controlled for months to years, clonidine may be tapered off and replaced by high doses of benzodiazepines, such as diazepam. Apart from these agents, experimental treatment of baroreflex failure includes (non-registered) inhibitors of norepinephrine release.

Agents that increase synaptic norepinephrine concentrations and may thereby elicit profound pressor responses are probably better avoided in baroreflex failure. These include tricyclic antidepressants, amphetamines, mono amino oxidase A inhibitors, cocaine, and tyramine-containing food and beverages. Additional nonpharmacological strategies include avoidance of individual factors that evoke sympathetic surges and (relaxation) biofeedback training. In the rare patients with predominant hypotension, low doses of fludrocortisone and increased dietary salt may be indicated. Regarding the polar shifts in blood pressure, treatment of baroreflex failure can be challenging and frequent follow-up of patients is warranted.

The clinical expression of baroreflex failure varies considerably among patients, probably depending on the extent of denervation of baroreceptors. In addition, the natural cause of baroreflex failure is unpredictable. In most cases, the pressor surges attenuate over time, but in some cases, volatile hypertension is permanent.

**CONCLUSION**

Baroreceptors are essential for buffering acute changes in blood pressure and prevent it from rising or declining excessively. The syndrome of baroreflex failure results from denervation of arterial and/or cardiopulmonary baroreceptors and is characterised by labile hypertension and rarely hypotension. Baroreflex failure is particularly likely to develop following carotid body tumour surgery, carotid artery surgery, neck irradiation and injury. Baroreflex failure should be considered in the differential diagnosis both of the cases with (severe) hypertension with a striking blood pressure variability and in patients with suspected pheochromocytoma. Referral to a specialised centre for correct diagnosis and specific therapy is warranted.

**REFERENCES**


ABSTRACT

HFE-related hereditary haemochromatosis is the most common autosomal recessive disorder in the Caucasian population. In 1996 the responsible gene (called HFE) was identified. Two mutations (C282Y and H63D) are considered most important and occur frequently in the Caucasian population.

We describe a family of an affected proband in which first- and second-degree relatives were tested phenotypically and genotypically. In second-degree relatives both C282Y homozygosity as well as compound heterozygosity were found. Family testing can be useful to detect persons who will possibly develop iron overload. We must be aware that testing first-degree relatives only carries a 2.5% chance that persons at risk of developing iron loading will not be detected. Cascade screening of second-degree relatives might be cost-effective.

INTRODUCTION

HFE-related hereditary haemochromatosis (HFErHH) is the most common autosomal recessive disorder in the Caucasian population, the prevalence ranging from 0.25 to 0.5%. A common trait of the disease is iron loading in tissues and organs such as liver, heart, joints, pancreas and pituitary gland. Ultimately this can lead to liver cirrhosis, cardiomyopathy, arthropathy, diabetes mellitus and hypogonadism.

In 1996 the gene responsible for HFE-related hereditary haemochromatosis (called HFE gene, coding for the HFE protein) was identified. The HFE protein is involved in the regulation of iron absorption. Multiple mutations in the HFE gene have been described. The most important one is the C282Y mutation, which means that at amino-acid 282 cysteine is substituted by tyrosine. A second (H63D, aspartate-to-histidine substitution at amino-acid 63) is also considered of importance.

The C282Y and H63D mutations occur frequently in the Caucasian population (see table 1). Among all Caucasian HFErHH patients 80 to 90% are homozygous for the C282Y mutation, 1% is homozygous for the H63D mutation, 5% are compound heterozygous (which means heterozygous for both the C282Y as well as the H63D mutation), and 3 to 10% are heterozygous for either C282Y or H63D mutation, possibly with other mutations.

Since the discovery of the HFE gene, it is possible to detect persons at risk of developing haemochromatosis in a presymptomatic stage. The Dutch Haemochromatosis Association advises testing of first-degree relatives of affected probands. In this original article, we will describe a family in which C282Y homozygosity and compound heterozygosity were found in second-degree relatives. The role and extent of genetic testing of families of affected probands will be discussed.

CASE REPORT

Patient A, 60 years old and diagnosed with diabetes mellitus nine years ago, presented with complaints of chronic fatigue. Physical examination did not reveal any abnormalities. Blood examination had the following results (reference range between brackets): haemoglobin 10.3 mmol/l (8.5-10.9), mean corpuscular volume 94 fl (80-100), aspartate aminotransferase 76 U/l (<40), alanine...
aminotransferase 79 U/l (<45), lactic dehydrogenase 976 U/l (<450), γ-glutamyl transpeptidase 216 U/l (<50), alkaline phosphatase 154 U/l (40-120), ferritin 1318 g/l (30-300), iron 39 g/l (14-28), and serum iron-binding capacity 55 g/l (45-77) and transferrin saturation (iron divided by iron-binding capacity) 71% (<45%). Ultrasound examination of the liver showed no abnormalities. Serological testing for infection with hepatitis A, B and C, Epstein-Barr virus and cytomegaly virus was negative. Liver biopsy showed iron loading with mild periportal fibrosis. The patient turned out to be C282Y homozygous.

Testing of family members
All first- and second-degree relatives of the patient were tested both phenotypically (ferritin level and transferrin saturation) and genotypically. The spouses of some of the first-degree relatives were tested as well. The results of this screening are shown in the family tree (figure 1) and table 2. Among the ten first-degree relatives one C282Y homozygote was found. Among ten second-degree relatives two compound heterozygotes and one C282Y homozygote were found.

DISCUSSION
HFE-related hereditary haemochromatosis seems to be a favourable disorder for screening. The disease has a long presymptomatic phase, an economical and simple treatment is available and – if treated in time – patients have the same life-expectancy as healthy persons.11 Two modalities exist for screening haemochromatosis: phenotypic testing (ferritin level and transferrin saturation) and genotypic testing (DNA examination). Phenotypic testing detects persons with elevated iron stores. However, phenotypic testing is a one-time measurement, so a normal test result does not exclude future iron loading. Genotypic testing detects persons who are at risk of developing iron overload, but not all detected persons will develop iron loading, indicating that HFE-related hereditary haemochromatosis is not a monogenetic disorder. Therefore, genotypic population screening for HFE-related hereditary haemochromatosis is still a matter of debate, because the clinical penetrance of the disorder (that means the percentage of persons with an ‘at risk’ genotype who will develop symptomatic iron loading) seems to be much lower than previously thought. Although C282Y homozygosity has a phenotypic expression (elevated ferritin and transferrin saturation) of 50 to 90%,12,13 the importance with respect to morbidity and mortality seems to be low. Beutler et al. found 152 C282Y homozygotes in a group of 41,038 healthy volunteers.13 Complaints and symptoms that could be ascribed to haemochromatosis were not more prevalent in these C282Y homozygotes than in a control group, so that the clinical penetrance in this study was only 1%.

In contrast to population screening, family testing is an accepted screening strategy to detect asymptomatic persons with iron overload. Detecting a C282Y homozygote with elevated iron stores implies phlebotomy therapy, whereas C282Y homozygotes without phenotypic expression should be re-tested phenotypically within several years. In contrast to C282Y homozygosity, the relevance of compound heterozygosity appears to be much lower.
since only 1 to 2% with this genotype develop a mild iron loading. As can be seen from figure 1 and table 2, genotype and phenotype often correlate poorly. However, the C282Y homozygote in the second-degree relatives (no. 20) was still young and it is possible that she will develop iron loading later in life. Furthermore, elevated iron parameters can be caused by conditions other than HFE-related hereditary haemochromatosis, as was recently discussed by Jacobs et al. The Dutch Haemochromatosis Association advises testing of first-degree relatives (siblings, children, parents) of C282Y homozygous patients both phenotypically and genotypically. In our patient second-degree relatives were tested as well. Among these second-degree relatives both C282Y homozygosity and compound heterozygosity were found, which means that spouses of first-degree relatives had introduced a C282Y and a H63D mutation into the family. As is the case in the general population, relatives of patients with HFE-related hereditary haemochromatosis with an ‘at risk’ genotype do not inevitably develop iron loading. However, in families the clinical penetrance of C282Y homozygosity appears to be higher than in the general population and is reported to be 40 to 67%. Bulaj et al. examined the penetrance in 214 C282Y homozygotes, including second-degree relatives, who were detected by family testing. From all men, 85% had an increased iron supply, 38% had signs and/or symptoms that could be ascribed to iron loading, including liver cirrhosis in 12%. However, a control group was not included in this study. Because of this higher clinical penetrance it can be questioned whether family testing for HFE-related hereditary haemochromatosis should include second-degree relatives. Extended family testing (first to third degree) has been advocated as an alternative to population screening, leading to detection of 40% of C282Y homozygotes. It is thought that the higher penetrance in families is caused by additional genetic and environmental factors that promote penetrance, although a search for such genetic factors was un rewarding. In second-degree relatives a dilution of these penetrance-promoting factors is expected. However, the magnitude and effect of this expected dilution is unknown so the penetrance in second-degree relatives is difficult to predict. A current two-year multicentre study of the clinical penetrance in first-degree relatives of Dutch HFE-related hereditary haemochromatosis patients will hopefully shed some light on this issue (www.zonmw.nl). If penetrance in these first-degree relatives turns out to be low, screening second-degree relatives will probably not be cost-effective. However, if the penetrance is in the reported range of 50%, a study of penetrance in second-degree relatives is needed. The chance of C282Y homozygosity in second-degree relatives of a C282Y homozygous index patient can be calculated as follows:

0.5 (= the chance of having a heterozygous sibling) x 0.1 (= the chance of a heterozygous spouse) x 0.25 (= the chance that both parents pass the mutation to their offspring) = 0.0125%
0.25 (= the chance of having a homozygous sibling) x 0.1 (= the chance of a heterozygous spouse) x 0.5 (= the chance that the spouse passes the mutation) = 0.0125%
Total = 0.025%

In this calculation C282Y heterozygosity of the parents of the index patient is assumed and the chance of a homozygous spouse is neglected.

At first sight this chance seems rather small. However, the yield of genetic testing in second-degree relatives can be enhanced by first testing the spouse of the first-degree relative (so-called cascade screening). If the sibling is C282Y heterozygous or homozygous, the spouse should be tested. The initial increase in costs and number of persons that need to be tested will be 75%. If the spouse carries no mutation, testing the children can be omitted. However, if the spouse is C282Y heterozygous (10% chance) there is at least a 25% chance of C282Y homozygosity in their offspring, which is the same as for siblings of the proband. The above-described approach proved to be cost-effective in screening children of affected probands. The difference in chance of C282Y homozygosity between children (5%)...

Table 2
Phenotype of the tested family members

| Family member | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
|---------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Iron          | 39 | 5  | 17 | 18 | 15 | 9  | 19 | 22 | 12 | 7  | 5  | 8  | 7  | 7  | 10 | 15 | 19 | 23 | 17 | 22 | 5  | 11 | 12 | 22 |
| TIBC          | 55 | 52 | 61 | 75 | 72 | 77 | 89 | 74 | 67 | 70 | 98 | 68 | 87 | 67 | 87 | 57 | 56 | 78 | 83 | 76 | 74 |
| Tf-saturation | 71 | 10 | 27 | 24 | 20 | 11 | 21 | 7  | 41 | 21 | 23 | 54 | 16 | 33 | 31 | 71 | 4  | 19 | 19 | 16 | 30 |
| Ferritin      | 1318 | 355 | 80 | 40 | 42 | 37 | 159 | 77 | 10 | 109 | 197 | 44 | 182 | 37 | 91 | 148 | 54 | 42 | 123 | 41 | 145 | 242 |
| Age           | 60 | 57 | 55 | 54 | 53 | 49 | 46 | 44 | 36 | 34 | 31 | 29 | 26 | 30 | 27 | 24 | 22 | 18 | 21 | 18 | 13 | 52 | 49 |

TIBC = total iron-binding capacity, Tf = transferritin, * not available.

Siezenga, et al. HFE-related hereditary haemochromatosis.
and second-degree relatives (2.5%) is rather small. However, in the aforementioned study the clinical penetrance in children was assumed to be 40%, whereas the penetrance in second-degree relatives is unknown. The findings of this study cannot thus be automatically extrapolated to screening second-degree relatives. It has been argued that detecting asymptomatic C282Y homozygotes would impose a psychological and economical burden upon these persons. Insurance denial has indeed been reported. However, a recent study showed that the quality of life and psychological well-being in asymptomatic C282Y homozygotes detected by screening is not different from unaffected persons.

In conclusion, testing second-degree relatives of patients with HFE-related haemochromatosis can be helpful in detecting persons who will possibly develop iron loading in the future. Testing first-degree relatives only implies a 2.5% chance that persons who are at risk for developing iron loading will not be detected. Testing second-degree relatives by means of cascade screening might be cost-effective. However, before recommendations on screening second-degree relatives can be made, studies to cost-effectiveness and penetrance are needed.

REFERENCES

A clinical and cardiovascular survey of Ehlers-Danlos syndrome patients with complete deficiency of tenascin-X


University Medical Centre St Radboud, Nijmegen, the Netherlands, tel.: +31 (0)24-361 37 24, fax: +31 (o)24-354 11 84, e-mail: j.schalkwijk@derma.umcn.nl, * corresponding author

ABSTRACT

Background: We recently described a new autosomal recessive type of Ehlers-Danlos syndrome (EDS) based on a deficiency of the extracellular matrix protein tenascin-X (TNX). TNX-deficient patients have hypermobile joints, hyperextensible skin and show easy bruising. Because of the reported cardiovascular abnormalities in other EDS types and the excessive haematoma formation after mild trauma in TNX-deficient individuals, we investigated whether cardiovascular or coagulation abnormalities occur in these patients.

Methods: We examined seven TNX-deficient patients. One of them had a mitral valve prolapse and died postoperatively after valve replacement, before the study was completed.

Results: Bleeding time and coagulation factors (INR, APTT, PT and fibrinogen) were all within the normal range. Ultrasonographic examination of the carotid and femoral arteries showed normal vessel wall compliance and distensibility. Echocardiography showed a slight billowing of the mitral valve in two patients from one family. All patients had normal diameters of aortic root and ascending aorta.

Conclusion: Although the patient group is small, there are no indications of generalised cardiovascular abnormalities in this type of EDS. We would recommend echocardiography for all these patients at the first evaluation and when a cardiac murmur appears.

INTRODUCTION

Ehlers-Danlos syndrome (EDS) is a heterogeneous group of inherited connective tissue disorders characterised by hyperextensibility of the skin, hypermobility of joints and tissue fragility which results in easy bruising and (in the classical type) atrophic scars following superficial injury. Six well-defined types of EDS have been described and the syndrome is generally regarded as a disorder of fibrillar collagen. Recently, we showed that deficiency of the extracellular matrix protein tenascin-X (TNX), encoded by the TNXB gene, causes a new type of EDS with autosomal recessive inheritance. Patients with a complete deficiency of TNX showed marked joint hypermobility, skin hyperextensibility and easy bruising, resembling the classical type of EDS. The six patients studied showed a variety of other clinical manifestations (such as spina bifida occulta, IgA deficiency, gastrointestinal bleeding, arteriosclerosis, goiter) although it is not clear whether these are causally related to TNX deficiency.

TNX deficiency is associated with abnormalities in collagen and elastic fibres, which are principal components of heart valves and large vessels. Cardiac abnormalities such as mitral valve prolapse and aortic dilatation are reported to be common features of certain EDS types, although this has been questioned in a more recent paper. Here we undertook an echocardiographic examination of six patients who were available for study and we investigated vessel wall properties of carotid and femoral arteries by ultrasonography. All these patients had excessive haematomas after relatively mild trauma or spontaneous haematomas. Although the most likely explanation for this phenomenon is vascular fragility due to connective tissue abnormalities, we wanted to exclude the possibility that the absence of serum TNX
caused abnormalities in coagulation. We therefore tested platelet function and performed screening coagulation tests.

**MATERIALS AND METHODS**

**Patients**
Six TNX-deficient patients were seen in our outpatient clinic. Three of them were identified through analysis of serum TNX levels in 151 Ehlers-Danlos patients. The other three were siblings. Initially we included seven patients; one of these patients died following cardiac surgery because of sepsis after a mitral valve replacement. These patients were described earlier.2

**Clinical features**
From the six patients medical history was recorded and physical examination was carried out with an emphasis on joint hypermobility, which was scored on a nine-point scale according to Beighton.1 An electrocardiogram was performed in all patients.

**Laboratory measurements**
Blood was taken for measurement of screening coagulation tests (INR, APTT, PT and fibrinogen). Platelet function was tested by means of Ivy, simplate and aggregation tests. Von Willebrand factor was measured as a marker for endothelial function.

**Echocardiography**
All patients underwent echocardiography, which was performed with standard equipment (GE ultrasound systems, Vingmed V, Horten, Norway, with second harmonic imaging (octave mode: transmit frequency of 1.7-1.9 MHz, receive frequency of 3.4-3.8 MHz)). Digitised measurements of the aortic root were taken in two-dimensional parasternal long-axis views at end diastole using the leading-edge technique at the four aortic levels described by Roman et al.:7 at the annulus, sinuses of Valsalva, supra-aortic ridge and proximal ascending aorta. Echocardiographic evidence of mitral valve prolapse consisted of severe bowing of the anterior and/or posterior mitral valve leaflet(s) into the left atrium and with the coaptation point of the leaflets on the atrial side of the mitral annulus.8 If the mitral valve leaflets showed bowing but the coaptation point was still on the ventricular side of the annulus or at the level of the annulus it was defined as ‘billowing’.

**Vessel wall compliance**
Cross-sectional compliance (CC) and distensibility coefficients (DC) are defined as the absolute and relative change in volume for a given change in pressure, respectively. Since arterial volume cannot be measured directly, simplified models have been used which assume that arteries have a circular shape and that the arterial length does not change during the cardiac cycle. With this simplification, CC and DC of separate arteries can be assessed by measuring diameters and diameter changes ultrasonographically using a vessel wall movement detector (Wall Track System, Maastricht, the Netherlands) as described by the group of Hoeks and Reneman.9,10 Measurements were performed by one person according to a protocol described by Van den Berkmortel et al.11 Traces were recorded at the following sites: 1) the right and left common carotid arteries (2 cm proximal of the bulb); 2) the right common femoral artery (at least 1 cm proximal of the bifurcation into the deep and superficial femoral artery).

**RESULTS**
All TNX-deficient patients had hyperextensible skin, hypermobile joints and easy bruising, as reported earlier. There were no signs of atrophic scarring. One of the patients had coexisting congenital adrenal hyperplasia. Three patients complained of recurrent (sub)luxation/dislocation of joints and two patients had chronic joint pain. One patient had a mild systolic murmur at the apex; the other patients had normal heart sounds. The electrocardiogram showed no abnormalities. Echocardiographic analysis showed no evident mitral valve prolapse, but two patients of the same family had billowing of the mitral valve. The diameter of the ascending aorta and aortic root was normal in all six patients available for study. Initially we included seven patients in this study. This seventh patient had a mitral valve prolapse that was complicated by a *Staphylococcus aureus* endocarditis in 1990. Mitral valve replacement was performed in July 2002. During surgery, the tissues of the patient appeared fragile and bled easily. Unfortunately, the patient died 16 days postoperatively due to sepsis. The mitral valve showed excessive tissue and a chorda rupture of the anterior mitral valve leaflet; microscopically there were myxoid degenerative changes. We did not observe fragmentaion and clumping of elastic fibres in this mitral valve as we recently found for the dermal elastic fibres in these patients.4 There were no signs of neovascularisation or inflammation. As large arteries are also rich in elastic fibres we examined the vessel wall compliance and distensibility of these arteries; these results were comparable with those of earlier measurements from healthy controls.12 Screening coagulation tests and platelet function tests were also normal.

**DISCUSSION**
There are several reports showing a high prevalence of cardiovascular abnormalities in EDS patients. Aortic rupture as seen in the vascular type of EDS is the most serious complication but also in the classical type of EDS cardiovascular complications were observed (Leier et al.)1 In

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hypermobility syndrome patients, Graham et al. found mitral valve prolapse to be more frequent in patients with a high hypermobility score, but echocardiography was only performed with the M-mode technique. A more recent study by Dolan et al. showed no evidence of increased frequency of mitral valve prolapse in patients with EDS. This apparent discrepancy can be explained by a possible bias in the study by Leier et al., because a substantial number of the reported patients in that study initially presented to a cardiologist. In our study, two patients of the same family had billowing of the mitral valve and one unrelated patient had a mitral valve prolapse. Because of the small patient group it is too preliminary to conclude that there is an association between TNX-deficient type EDS and cardiac abnormalities. In addition, billowing and prolapse of the mitral valve are not uncommon in the general population. Studies show a prevalence of 1 to 2%.

At this point, no general recommendation can be made as to whether all EDS patients should be subjected to echocardiography. Former studies show that cardiac abnormalities and aortic root dilatation occur in all subtypes of EDS but studies on the prevalence of cardiac abnormalities within the specific EDS subtypes and studies concerning long-term outcome of the cardiovascular abnormalities have not been carried out. The EDS type based on TNX deficiency described here can easily be discriminated from the other types, because of the absence of atrophic scarring and family history. We would recommend echocardiography for these patients at the first evaluation and when a cardiac murmur appears. If there is a mitral valve prolapse with coexisting mitral insufficiency patients should be followed yearly or every other year according to the severity of the disorder. In case of interventions patients should be treated according to the endocarditis prophylaxis.

We recently found that dermal elastic fibre structure is disturbed in TNX-deficient patients. Whether these patients suffer from a more generalised elastinopathy remains to be investigated. Our measurements of arterial vessel wall mechanical properties showed no evident abnormalities nor did we observe abnormal elastic fibres in the mitral valve that was available for study. In large blood vessels there is co-expression of TNX and tenascin-C (TNC), which is another member of the tenasin gene family. TNC could possibly compensate for the absence of TNX leading to normal vessel wall properties.

One of the clinical features of the TNX-deficient patients is easy bruising. This could be caused by mechanical fragility of the vascular connective tissue. Since a 140 kDa TNX fragment is present in plasma, this could theoretically affect the intrinsic coagulation properties. We found, however, no impaired coagulation in these patients.

In conclusion, we would recommend echocardiography for patients with EDS due to TNX deficiency, at the first evaluation and when a cardiac murmur appears.

References


Peeters, et al. Ehlers-Danlos syndrome patients with complete deficiency of tenasin-x.
PHOTO QUIZ

A 72-year-old patient with diarrhoea and abdominal pain

T.S. van der Werf*, J.G. Zijlstra, R.F.E. Wolf

Intensive and Respiratory Care Unit, Departments of Internal Medicine and Radiology, Groningen University Medical Centre, PO Box 30001, 9700 RB Groningen, the Netherlands, e-mail: t.s.van.der.werf@int.azg.nl, * corresponding author

CASE REPORT

A 72-year-old diabetic male was referred by his general practitioner because of gradually increasing diarrhoea and abdominal pain over several days. On examination, he had mild acidic breathing, and he appeared slightly dehydrated. The abdomen was distended and tender on palpation. Blood chemistry revealed slight derangement of his diabetes, and a neutrophil count of 12 x 10⁹/l, liver enzymes were slightly elevated (ASAT 60, ALAT 70 U/l). During clinical observation, symptoms worsened, and a plain abdominal radiograph was performed (figure 1).

WHAT IS YOUR DIAGNOSIS?

See page 175 for the answer to this photo quiz.

Figure 1
Plain abdominal (antero-posterior) radiograph in supine position
Extreme leucocytosis and splenomegaly in metastasised melanoma

J.F. de Wolff¹, E.V. Planken¹, G.J. den Ottolander²

¹Department of Internal Medicine, Diaconessenhuis, Leiden, the Netherlands, ²Department of Haematology, Leiden University Medical Centre, Leiden, the Netherlands, * corresponding author: 38 Bullescroft Road, Edgware, Middlesex HA8 8RW, United Kingdom, e-mail: jfdwolff@doctors.org.uk

CASE REPORT

A 63-year-old woman was referred to the internist because of progressive fatigue and breathlessness for several months. She even got breathless from talking. She had lost 15 kg of weight and had intermittent fevers up to 38.6°C. There were no night sweats. Several courses of oral broad-spectrum antibiotics had not had any effect on her symptoms. The previous medical history was unremarkable apart from an unspecified lung operation at the age of 13 and chronic obstructive pulmonary disease. Ten months earlier a melanoma had been removed from her left ankle, histologically Breslow depth 1.5 mm and Clarke level III. Her medication consisted of inhaled bronchodilators, steroids and acetylcysteine. The family history included a brother with multiple sclerosis and a brother with colonic carcinoma. On physical examination a pale and breathless woman was seen. She was 1.65 m tall and her weight was 60.3 kg. The pulse was 100 beats/min, blood pressure 120/70 mmHg and rectal temperature 39.0°C. There were no enlarged lymph nodes and no pathological findings of heart and lungs. No breast lumps were palpable. Her liver was palpable under the right costal margin and the spleen was palpable 10 cm under the left costal margin. There was no peripheral oedema and no other findings at the extremities. Laboratory findings were: Hb 5.5 mmol/l (8.91 mg/dl), thrombocyte count 377 x 10⁹/l, leucocyte count 62.4 x 10⁹/l (differential: neutrophils 73%, eosinophils 11%, basophils 0%, lymphocytes 11%, monocytes 4%, some metamyelocytes and myelocytes; erythroblasts were not observed). The electrolytes and renal function were normal. Liverfunction tests: ASAT 21 U/l, ALAT 12 U/l, LDH 527 U/l, alkaline phosphatase 295 U/l, γGT 196 U/l, ferritin 379 μg/l and serum iron 3.1 μmol/l, CRP was elevated at 101 mg/l. Bone marrow biopsy showed hypercellular bone marrow with all cell lines represented, though a strong increase in granulopoiesis was seen. Occasionally some very atypical cells were seen with polymorphic hyperchromatic nuclei, a coarse chromatin pattern and enlarged nucleoli. Bone marrow cytology showed an increased granulopoiesis (70%) with moderate left shift, 2% blasts, increase of eosinophilic elements (14%) with multiple precursors. There was decreased erythropoiesis and megakaryopoiesis. Scattered through the marrow there were large, very atypical cells with an epithelioid aspect, one or more round to oval nuclei, abundant cytoplasm which sporadically contained blue-grey granula (see figure 1). These cells were identified as (amelanotic) melanoma cells.

ABSTRACT

A 63-year-old woman presented to the internist with fatigue, cough, low-grade fever, splenomegaly and leucocytosis up to 130 x 10⁹/l. Although a diagnosis of chronic myelogenous leukaemia was initially entertained, she turned out to have a metastasised melanoma. The differential diagnosis and workup is discussed, as well as potential mechanisms by which the tumour could have induced the leucocytosis, such as the production of G-CSF or similar mediators, and the prognostic significance of this phenomenon.

Extreme leucocytosis and splenomegaly in metastasised melanoma

J.F. de Wolff¹, E.V. Planken¹, G.J. den Ottolander²

¹Department of Internal Medicine, Diaconessenhuis, Leiden, the Netherlands, ²Department of Haematology, Leiden University Medical Centre, Leiden, the Netherlands, * corresponding author: 38 Bullescroft Road, Edgware, Middlesex HA8 8RW, United Kingdom, e-mail: jfdwolff@doctors.org.uk

CASE REPORT

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Cytogenetics showed 20 cells with a normal female karyotype and no \textit{bcr-abl} fusion on fluorescent in situ hybridisation (FISH) in ten metaphases. Blood cultures (two separate 3-10 ml aliquots containing aerobic and anaerobic media) were negative. Mid-stream urine examination showed several leucocytes per high-power field and upon culture contained mixed bacterial flora. Sputum was negative for auramine and Ziehl-Neelsen staining but on culture showed \textit{Haemophilus parainfluenzae}.

Ultrasound examination of the abdomen showed an enlarged liver with multiple lucencies, a very large spleen of 20 cm with one lucency possibly due to metastasis, some ascites and no enlarged lymph nodes. Computed tomography (CT) of chest and abdomen showed no signs of a primary tumour, signs of infiltration in the left lower pulmonary lobe, many metastases in the liver and a solitary one in the spleen, one pathological lymph node around the abdominal aorta and some ascites. Ultrasound-guided biopsy of one of the liver lesions showed disseminated melanoma cells in small clusters and marked granulocytosis of the sinuses. Immunohistochemical staining was positive for melanoma (HMB-45, S100, Schmorl’s stain weak).

During the stay in hospital the leucocytosis increased to $130.3 \times 10^9/l$ despite antibiotic therapy and the patient’s condition worsened with night sweats and progressive fatigue. She declined chemotherapy, and was released with terminal home care; she died at home one day after discharge. A postmortem examination was not performed.

**DISCUSSION**

Extreme leucocytosis can be caused by infection, malignancy and several other, less common causes. When leucocyte counts exceed $50 \times 10^9/l$ it is referred to as ‘leukaemoid reaction’ (see table 1). Reding et al. studied 100 patients presenting with leucocytosis exceeding $25 \times 10^9/l$ and found infection as a cause in only 48%.\(^1\) In 15% malignancy was diagnosed and in the remaining 37% other, less common secondary causes were found (such as haemorrhage, glucocorticoid therapy, rhG-CSF therapy).

**Table 1**

\textbf{Causes of neutrophilic leukaemoid reaction (leucocyte count $>50 \times 10^9/l$)\(^{15}\)}

\begin{itemize}
  \item **INFECTIONS**
    \begin{itemize}
      \item Pneumonia
      \item Meningitis
      \item Diphtheria
      \item Tuberculosis
      \item Shigellosis
    \end{itemize}
  \item **INTOXICATIONS/MEDICATION**
    \begin{itemize}
      \item Mercury poisoning
      \item Steroids
      \item Adrenergic agents
      \item All-trans retinoic acid (ATRA)
      \item G-CSF
    \end{itemize}
  \item **PHYSICAL CAUSES**
    \begin{itemize}
      \item Serious burns
    \end{itemize}
  \item **MALIGNANCY**
    \begin{itemize}
      \item Bone metastasis
      \item Multiple myeloma
      \item Myelofibrosis
      \item Hodgkin’s disease
      \item Other malignancy
    \end{itemize}
  \item **SERIOUS HAEMORRHAGE**
  \item **ACUTE HAEMOLYSIS**
  \item **ECLAMPSIA**
  \item **PREMATURITY/NEONATES**
  \item **HEREDITARY/CONGENITAL**
\end{itemize}
The leucocyte count was not predictive of the cause. The 15 patients with malignancy mostly had primary haematological disorders, but metastatic carcinoma was diagnosed in six of them.

Fatigue, splenomegaly and leucocytosis as presenting signs in a middle-aged patient all point at the possibility of myeloproliferative syndrome (MPS). However, careful review of the history, physical examination, infectious parameters (such as blood culture, chest X-ray and mid-stream urine specimen), blood film and bone marrow aspirate and trephine can give clues as to a secondary cause.

After excluding infectious disease and drug-related causes of leucocytosis, the diagnostic effort should concentrate on the possibility of malignancy. To differentiate between primary haematological and secondary causes, normal to low basophil granulocytes on the blood film, as well as negative cytogenetics (no t(9;22)(q34;q11) translocation causing bcr-abl fusion) make myeloproliferative disease very unlikely.

There are roughly two ways in which nonhaematological malignant disease can lead to leucocytosis. Firstly, bone marrow invasion by metastases can cause a leucoerythroblastic picture. Secondly, an oft-reported form of leucocytosis in malignancy is a leukaemoid reaction due to production of cytokines or other mediators by tumour cells.

In our patient, infectious causes were considered unlikely, with blood cultures remaining sterile all through the patient's stay in hospital. She was not taking any oral medication associated with a raised leucocyte blood count. The absence of bcr-abl fusion on cytogenetics and the normal basophil count made the diagnosis of chronic myelogenous leukaemia (CML) very unlikely (<5% of CML patients are bcr-abl negative). The finding of metastatic lesions of the primary melanoma in the liver biopsy, as well as malignant cells in the bone marrow, confirmed the suspected diagnosis of tumour-induced leucocytosis. Bone marrow infiltration as such was not perceived to be an explanation for the symptoms, given the absence of leucoerythroblastosis in the blood. Therefore, the diagnosis was leukaemoid reaction due to the production of a humoral factor by a primary melanoma.

Virtually every type of malignant tumour has been reported to induce leucocytosis, for example glioblastoma multiforme, head and neck carcinoma and malignant mesothelioma. Generally the secretion of G-CSF or GM-CSF, most commonly the former, by the tumour cells seems to be the predominant mechanism of the leukaemoid reaction in malignancy.

Malignant melanoma has been reported to secrete G-CSF or GM-CSF, and this feature appears to be a marker of poor prognosis. In the patient reported by Carey and Kunz the leucocytosis resolved when the adrenal metastases were removed through bilateral adrenalectomy. Eosinophilic leukaemoid reaction has been reported in a case of melanoma secreting extremely high quantities of IL-5.

Concerning the contribution of G-CSF production to a tumour's malignant potential, Safarians et al. found that G-CSF-secreting melanoma cells do not express G-CSFR, thereby ruling out an autocrine mechanism. Otherwise, G-CSF can stimulate endothelial proliferation and migration, thereby assuring the tumour of a steady blood supply through angiogenesis. Moreover, the granulocytes themselves can benefit the tumour by inducing further malignant transformation.

Finally, Safarians et al. suggest that the expression of G-CSF could be a side effect of the activation of other oncogenes, which increase the tumour's capability of malignant transformation, growth and dissemination.

**CONCLUSION**

The diagnosis in this patient turned out to be metastasised melanoma with extreme granulocytosis, possibly due to G-CSF production by the tumour cells.

**ACKNOWLEDGEMENT**

Dr M.C.B. Gorsira, Diaconessenhuis Leiden, for providing the liver biopsy slide.

**REFERENCES**

ABSTRACT

We describe a 61-year-old patient who had been suffering from chronic ulcers of both legs for 18 months. Initially, his condition was diagnosed as ischaemic because of an ankle-brachial index of 0.6, as confirmed by additional angiography. A successful femoro-infragenual bypass procedure was performed, but the ulcers increased in size and number. He was then extensively analysed for a possible (macro)vascular origin of his symptoms. Angiographic analysis of both legs showed no arterial stenosis or occlusion. Despite the extensive experience of the vascular surgeons with leg ulcers, consultations by internal medicine, vascular medicine and dermatology, and tissue examination by our pathologists, pyoderma gangrenosum was not recognised. During a multidisciplinary meeting one of the specialists, to whom the lesions were shown, immediately considered the diagnosis on clinical grounds. The additional finding of IgG-κ paraproteinaemia and improvement of the ulcers on treatment with corticosteroids were consistent with the diagnosis. Although the majority of patients on the vascular surgery ward have ulcers caused by ischaemia or a combined arterial/venous origin, another (rare) cause, namely pyoderma gangrenosum in association with IgG-κ paraproteinaemia without the presence of multiple myeloma, should be taken into account.

INTRODUCTION

Pyoderma gangrenosum (PG) is an uncommon, idiopathic, chronic ulcerative inflammatory skin disease. Four types of PG have been recognised: the ulcerative, pustular, bullous and vegetative types. The first type is characterised by a severely painful ulceration surrounded by an erythematous halo. In the majority of the affected patients there is an underlying condition, usually inflammatory bowel diseases (such as ulcerative colitis, Crohn’s disease and diverticular disease), arthritis (such as rheumatoid arthritis, spondylarthropathy), chronic hepatitis and haematological malignancy (including acute and chronic myeloid leukaemia, myelofibrosis, lymphoma) and solid tumours of the colon, bladder, prostate, breast, bronchus, ovary, and adrenocortical carcinoma. This type is commonly located on the lower limb, but location on the trunk, penis, head and neck, breast, and ocular sites have also been reported. The ulcerative form of PG typically affects people in the age range 25 to 55 years. The second type is also painful with pustules again surrounded by an erythematous halo. This condition is usually associated with inflammatory bowel disease (ulcerative colitis) and rarely with polycythaemia rubra vera, hepatobiliary disease and pyostomatitis vegetans. The third type (also designated as atypical PG) is characterised by painful vesicles, with a tendency to enlarge rapidly in waves with central necrosis and erosion with an erythema as a surrounding halo. This type is usually present in

Symmetrical, painful ulceration of the lower limbs in a vascular surgery ward: a diagnostic challenge

Pyoderma gangrenosum associated with IgG-κ paraproteinaemia

S. Simsek1*, R.F.F. van den Haak2, A.E. Voskuyl3, J.A. Rauwerda2, R.J.M. Strack van Schijndel4

Departments of 1Endocrinology/Diabetes Centre, 2Surgery, 3Rheumatology and 4Internal Medicine, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, the Netherlands, tel.: +31 (0)20-444 43 07, fax: +31 (0)20-444 43 13, e-mail: Simsek@vumc.nl, * corresponding author

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combination with haematological dyscrasias. Confusion with Sweet’s disease (acute febrile neutrophilic dermatosis) can easily occur. Sweet’s disease is very uncommon and characterised by uncomfortable chronic erythematous plaques with sinus discharge. Other disorders associated with PG include HIV, hepatitis C, thyroid disease, diabetes mellitus, cryoglobulinaemia, lupus erythematosus, dermamyositis, sarcoidosis, vasculitis (Wegener’s granulomatosis, Takayasu’s arteritis, Behçet’s disease) and paroxysmal nocturnal haemoglobinuria (table 1).

The association of the ulcerative type of PG with benign monoclonal gammopathy, especially of immunoglobulin A (IgA), has also been described. We report a patient suffering from the ulcerative type of PG of both legs, which was found to be associated with IgG-κ paraproteinaemia.

CASE REPORT

A 61-year-old patient was admitted to the department of vascular surgery because of chronic ulcers of both legs (for 18 months). These ulcers were initially identified as ischaemic lesions (an ankle-brachial index of 0.6) and femoro-infragenual bypass surgery was performed. Soon after this operation, he developed more painful ulcers, despite normalisation of the ankle-brachial index. Moreover, subsequent angiographic analysis of the arteries of the legs showed an open bypass without any indication for (macro)vascular stenosis/occlusion. Duplex examination did not show venous occlusion either. The patient had been treated with several antibiotics, without any improvement in the clinical situation. Then he was send to our university hospital. Cardiovascular risk factors, including hypertension and dyslipidaemia, were adequately controlled. He was not complaining of headache, fever or changes in bowel movement. Further medical history was unremarkable. Physical examination revealed a normal blood pressure, normal temperature, lack of tenderness of the temporal arteries, and large ulcerations involving the pretibial area of both legs, with blood and pus in the centre and a region of deep necrosis (figure 1A). Laboratory data disclosed an elevated erythrocyte sedimentation rate (100 mm/h), Hb 6.2 mmol/l, MCV 82 fl, leucocytosis 11.9 x10⁹/l with 90% neutrophils, normal platelet count, slightly elevated creatinine level 152 µmol/l, and a normal calcium. Thyroid function was normal. Tests for hepatitis B, C and lues were negative. Antinuclear antibodies, rheumatoid factor, antineutrophil cytoplasmic antibodies and antiphospholipid antibodies were absent. A chest radiogram and a computer tomography of the abdomen revealed no abnormalities. A skin biopsy revealed lymphocytic as well as neutrophilic inflammation. Moreover, the biopsy showed plasma cells, necrosis and re-epithelialisation. Vasculitis was absent in the biopsy.

Table 1

<table>
<thead>
<tr>
<th>Diseases associated with pyoderma gangrenosum</th>
</tr>
</thead>
</table>

**INFLAMMATORY BOWEL DISEASE**
- (Chronic) ulcerative colitis
- Crohn’s disease
- Diverticular disease
- Regional enteritis

**ARTHRITIS**
- Seronegative with inflammatory bowel disease
- Seronegative without inflammatory bowel disease
- Rheumatoid arthritis
- Spondylarthropathy

**HAEMATOLOGICAL DISEASE**
- (Acute and chronic) myelocytic leukaemia
- Monoclonal gammopathy (IgA)
- Hairy cell leukaemia
- Myelofibrosis
- Polycythaemia rubra vera
- Lymphoma: Hodgkin’s disease, non-Hodgkin’s disease, cutaneous T cell

**IMMUNE ABNORMALITIES**

<table>
<thead>
<tr>
<th>Humoral</th>
<th>Congenital and acquired hypogammaglobulinaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Selective, complete and hyperimmunoglobulin E syndrome</td>
</tr>
<tr>
<td></td>
<td>Streaking leucocyte factor</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell-mediated</th>
<th>Defective neutrophil function: reduced chemotaxis and impaired phagocytosis and oxygen uptake, aberrant neutrophil trafficking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abnormal monocyte function</td>
</tr>
<tr>
<td></td>
<td>Abnormal response to skin antigens</td>
</tr>
<tr>
<td></td>
<td>Congenital deficiency in leucocyte-adherence glycoproteins</td>
</tr>
<tr>
<td></td>
<td>Immunodeficiency/immunosuppression</td>
</tr>
</tbody>
</table>

**SOLID TUMOURS ASSOCIATED WITH PG**
- Colon, bladder, prostate, breast, bronchus, ovary, adenocortical carcinoma

**DRUGS TRIGGERING PG**
- Alpha 2b-interferon

**OTHERS**
- Chronic active hepatitis, cryoglobulinaemia and hepatitis C, thyroid disease, pulmonary disease, hirudinidisis suppurativa, acne conglobata, sarcoidosis, atrophic gastritis, diabetes mellitus, systemic lupus erythematosus, Takayasu’s arteritis, dermatomyositis, HIV, Wegener’s granulomatosis, sensorineural deafness, paroxysmal nocturnal haemoglobinuria, peripheral ulcerative keratitis, lung injury, primary biliary cirrhosis
Initially a definitive diagnosis could not be made. During a multidisciplinary meeting, however, one of the specialists, to whom the lesions were shown, immediately considered the diagnosis pyoderma gangrenosum on clinical grounds. Further laboratory analysis, i.e. serum protein electrophoresis, showed an IgG-H9260 paraproteinaemia. To see whether there was a systemic skeletal involvement additional radiograms were performed, without signs of multiple myeloma. Moreover, neither cytology nor histology of the bone marrow showed a localisation for multiple myeloma. The ulcers of both legs started to heal within a week after treatment with prednisolone 1 mg/kg was initiated (figure 1B). Altogether, the presence of the monoclonal gammopathy and the improvement of the lesions after treatment were consistent with PG.

**DISCUSSION**

Pyoderma gangrenosum in association with IgG-H9260 paraproteinaemia is uncommon.10-12 Although the association with monoclonal IgA gammopathy is well recognised, multiple myeloma is rarely found. Also in our patient, we could not find signs of systemic skeletal involvement. The conditions that may mimic ulcerative PG are systemic vasculitis, dermatitis artefacta, infection (mycobacterial, atypical mycobacterial, amebiasis, syphilis, herpes simplex, deep fungal infection), drug reactions (hydroxyurea), insect bite (spider), synergistic gangrene, antiphospholipid syndrome and cutaneous neoplasma (table 2). PG is a diagnosis of exclusion, so the above-mentioned conditions should be ruled out before the diagnosis of PG is made. Weenig et al. have described that substantial misdiagnosis of skin ulcers as PG can occur (approximately 10%).13 Misdiagnosis of PG can expose patients to risks associated...
with its treatment. In that study, 95 patients appeared to have skin ulcers caused by other diseases, although initially PG was suspected. These causative diseases were vascular occlusive or venous disease (such as antiphospholipid syndrome, venous stasis ulceration and type I cryoglobulinaemia), vasculitis (among them Wegener’s granulomatosis, polyarteritis nodosa and cryoglobulinaemic (mixed) vasculitis), cutaneous involvement of malignant process (usually lymphoma), primary cutaneous infection (such as deep fungal infection, herpes simplex virus type 2, cutaneous tuberculosis), drug-induced or exogenous tissue injury (among them Münchausen’s syndrome/factitial disorder, hydroxyurea-induced ulceration). The authors thus stress the need to follow a thorough diagnostic evaluation, including collecting careful medical historical data, being aware of characteristic features on physical examination, performing a skin biopsy for not only histopathological purposes but also for tissue culture, and ordering laboratory investigations with the aim of ruling out diagnoses that mimic PG (such as erythrocyte sedimentation rate, complete blood count, blood chemistry, protein electrophoresis, chest radiography, antiphospholipid, antinuclear, and antineutrophil cytoplasmic antibodies). When PG is eventually established as the diagnosis, the patient should be evaluated thoroughly to rule out other diagnoses after long-term follow-up.

However, in our patient, the opposite of what Weenig et al. described in their study population happened. Namely, it took rather a long time before a definitive diagnosis of PG was made, due to the other possible causes of the ulceration. Skin biopsies in all forms of PG are characterised by a central necrosis accompanied by a massive peripheral neutrophilic infiltration and perivascular and intramural lymphocytic infiltrates. A possible pathogenetic mechanism underlying the association between PG and monoclonal gammopathy could be the defective monocyte function, as was suggested by Norris et al. and Jones et al. Other possible mechanisms include abnormality of neutrophil and monocyte chemotaxis, phagocytosis (including defective leucocyte adherence glycophorins), mast cell activation, hypogammaglobulinaemia or association with hyperimmunglobulinaemia E. Crowson et al. mention in their review article that the pathogenetic mechanism and the initial trigger are dependent on the associated/underlying systemic disease, eventually leading to the endpoint of a neutrophilic dermopathy with diverse destruction of the epidermis and the adnex. In conclusion, we report a patient with chronic ulcers of both legs with a history of peripheral bypass surgery and (cardio)vascular risk factors. Despite an extensive search for an ischaemic aetiology, a rare diagnosis, pyoderma gangrenosum associated with IgG-κ paraproteinaemia, was found to be the cause of the lesions. This case illustrates that the diagnosis of pyoderma gangrenosum is not easy: many specialists were involved before one of them coined the possibility of a PG. Since the disease is rare, recognition is difficult. Suspicion of PG should be raised when ulcers are present on the lower legs that are painful and symmetrical and for which there is no other obvious explanation. A thorough diagnostic effort must be made to reveal underlying disease when PG is diagnosed.

ACKNOWLEDGEMENT

We would like to thank dr. F. stam for his help.

REFERENCES

Recurrence acute pancreatitis after isoniazid

K.M. Chow*, C.C. Szeto, C.B. Leung, P.K.T. Li

Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong, SAR, China, tel. +852-2632 31 31, fax: +852-2637 53 96, e-mail: Chow_Kai_Ming@alumni.cuhk.net, *corresponding author

ABSTRACT

Drug-induced acute pancreatitis should be in the differential diagnosis of acute abdomen occurring soon after initiation of tuberculosis treatment and chemoprophylaxis. Isoniazid-induced pancreatitis is potentially reversible; early recognition and drug withdrawal are warranted in the appropriate clinical setting. We present a case of reversible acute pancreatitis after isoniazid treatment of genitourinary tuberculosis, followed by recurrence of pancreatitis 12 years later when the patient received isoniazid again for pulmonary tuberculosis. Isoniazid-induced pancreatitis, if highly suspicious or confirmed with re-challenge test, mandates permanent avoidance of the drug.

INTRODUCTION

Isoniazid has been used for treatment of Mycobacterium tuberculosis for over 50 years. Although it was initially considered to be free of serious side effects, the potential adverse effect of isoniazid hepatotoxicity has now become evident. Furthermore, as our experience with isoniazid accumulates, there have been more reports of isoniazid-induced pancreatitis in post-marketing surveillance. This category of adverse effect, albeit less frequent than hepatotoxicity, should be promptly recognised because development of acute pancreatitis mandates drug withdrawal and permanent isoniazid avoidance as illustrated in our case.

CASE REPORT

A 25-year-old Chinese patient with a history of systemic lupus erythematosus presented with malaise and progressive uraemic symptoms. There was no history of alcohol abuse. Plasma creatinine was 18.0 mg/dl (normal range, 0.6 to 1.4 mg/dl). Urinary sediment was unremarkable except for sterile pyuria. Advanced renal failure with quiescent lupus nephritis was diagnosed, followed by identification of acid-fast bacilli in the early morning urine. Intermittent peritoneal dialysis was commenced after Tenckhoff catheter insertion, together with implementation of quadruple tuberculosis treatment consisting of isoniazid 200 mg daily (5.5 mg/kg/day), rifampicin, pyrazinamide and ethambutol.

The patient developed acute severe pain in the epigastrium region three weeks after initiation of tuberculosis therapy, before the scheduled date for continuous ambulatory peritoneal dialysis training. Examination revealed diffuse abdominal tenderness. His serum and peritoneal dialysate amylase levels were 2071 U/l (normal range, 44-128 U/l) and 1478 U/l, respectively. An erect chest radiograph demonstrated right apical granuloma only. Abdominal sonography and computed tomographic imaging showed an oedematous pancreas, in the absence of biliary tract disease. The clinical picture of acute pancreatitis resolved with conservative management and withdrawal of isoniazid therapy. There were no other identifiable causes of acute pancreatitis.

Subsequent cadaveric kidney transplantation was complicated by progressive allograft dysfunction secondary to chronic rejection. Twelve years later, reactivation of tuber-
culosis occurred with pulmonary involvement. When isoniazid 200 mg daily, rifampicin and levofloxacin were started, his plasma creatinine was 6.7 mg/dl with an estimated glomerular filtration rate of 12 ml/min/1.73 m².

After three weeks of tuberculosis therapy, the patient presented acutely with marked epigastric tenderness. The serum amylase level rose from 429 U/l to 2100 U/l within five days. He had been receiving prednisolone, cyclosporin and azathioprine for more than ten years, and the serum amylase levels were normal in between the two attacks. Ultrasound imaging confirmed the clinical impression of acute nonbiliary pancreatitis. Again, the pain resolved 72 hours after discontinuation of isoniazid, accompanied by biochemical and radiological evidence of improvement. No further bouts of pancreatitis were encountered thereafter.

**DISCUSSION**

Drug-related acute pancreatitis is uncommon. As always, causal relationship based on anecdotal case report(s) has been a matter of controversy. Theoretically, it should be clearly documented that pancreatitis developed during treatment with the implicated drug, was reversed by withdrawal of the drug, and recurred when the drug was reintroduced. We have previously reported another end-stage renal disease patient with isoniazid-induced pancreatitis, which was supported by means of re-challenge test. In case of tuberculosis therapy, controlled re-challenge with the drug suspected of causing pancreatitis is deemed necessary in view of the limited range of drugs available for *Mycobacterium tuberculosis* infection as well as the confounding drug culprit rifampicin. Now considered the first-line essential antituberculous drug, isoniazid should be included in all tuberculosis treatment regimens unless contraindicated.

According to the case reports in literature, acute pancreatitis associated with isoniazid develops within three weeks after drug administration and consistently recurs, with a much earlier onset, if re-challenged soon after resolution of pancreatitis. The role of concurrent rifampicin may not be directly contributory since this complication has been documented in several cases of isoniazid monotherapy (mostly chemoprophylaxis therapy). It remains unclear whether the reaction of isoniazid-induced pancreatitis occurs in a dose-dependent manner or in the setting of a hypersensitivity syndrome to isoniazid. Given the short time lapse (i.e. hours between re-challenge and occurrence of pancreatitis), the latter is most likely. The current case allows us to evaluate recurrence of pancreatitis as isoniazid was re-introduced 12 years after the initial episode. Irrespective of the mechanisms of isoniazid-induced pancreatitis, the message here is clear: isoniazid-induced pancreatitis occurs in a dose-dependent manner or in the setting of a hypersensitivity syndrome to isoniazid.

Table 1

<table>
<thead>
<tr>
<th>REFERENCE</th>
<th>SUBJECT</th>
<th>SEX/AGE</th>
<th>ESRD</th>
<th>TREATMENT DOSE (MG)</th>
<th>DAILY</th>
<th>DIAGNOSIS</th>
<th>AMYLASE IN SERUM (U/L)</th>
<th>ONSET (DAY(S)) OF PANCREATITIS AFTER START OF ISONIAZID</th>
<th>ONSET OF PANCREATITIS AFTER RE-CHALLENGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>F/43</td>
<td></td>
<td>300</td>
<td>Chemoprophylaxis, sarcoidosis</td>
<td>Not done</td>
<td>4</td>
<td>2 hours</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>M/31</td>
<td></td>
<td>300</td>
<td>Gastrointestinal tuberculosis</td>
<td>738</td>
<td>14</td>
<td>6 hours</td>
<td></td>
<td></td>
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<tr>
<td>6</td>
<td>M/68</td>
<td></td>
<td>300</td>
<td>Urinary bladder treatment</td>
<td>458</td>
<td>0.5</td>
<td>No re-challenge</td>
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<td>1</td>
<td>M/31</td>
<td>+</td>
<td>300</td>
<td>Pulmonary tuberculosis, IgA nephropathy</td>
<td>1672</td>
<td>21</td>
<td>5 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M/80</td>
<td>+</td>
<td>300</td>
<td>Tuberculous spondylitis, ischaemic nephropathy</td>
<td>782</td>
<td>2</td>
<td>No re-challenge</td>
<td></td>
<td></td>
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<tr>
<td>8</td>
<td>M/42</td>
<td></td>
<td>300</td>
<td>Tuberculous spondylitis</td>
<td>645</td>
<td>11</td>
<td>8 hours</td>
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<tr>
<td>9</td>
<td>F/28</td>
<td></td>
<td>300</td>
<td>Chemoprophylaxis</td>
<td>336</td>
<td>21</td>
<td>No re-challenge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Our case report</td>
<td>M/25</td>
<td>+</td>
<td>200</td>
<td>Genitourinary tuberculosis, systemic lupus erythematosus</td>
<td>2071</td>
<td>21</td>
<td>21 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ESRD = end-stage renal disease, F = female, M = male.
REFERENCES

DIAGNOSIS

The plain abdominal radiograph reveals what was suspected during the repeat physical examination: a caecal blow-out. The radiograph shows gas around the pathologically distended caecal part of the colon, and gas collection anterior and medial of the caecum.

The computed tomography (figure 2) confirmed this diagnosis, and caecal perforation with early peritonitis was found during laparotomy. The postoperative course was complicated by multiple organ failure. The patient was transferred to our intensive care unit. Despite fluid resuscitation, mechanical ventilation, vasopressor and antimicrobial treatment, and renal replacement therapy, he died from refractory septic shock.

The cause of his diarrhoea was not established. In hospital populations, caecal perforation is a well-known complication of Clostridium difficile toxin-mediated diarrhoea. Several infectious conditions have occasionally been associated with diarrhoea, toxic megacolon and caecal perforation. These infections include bacteria (Salmonella spp, tuberculosis), helminths (Schistosoma mansoni) and viruses (CMV). Besides, collagen-vascular diseases such as Wegeners' granulomatosis may occasionally cause a similar clinical pattern. Idiopathic colonic pseudo-obstruction (Ogilvie's syndrome) may also cause caecal blow-out.

REFERENCES


Figure 2
Abdominal CT confirms the presence of gas outside the caecal lumen that is clearly distended

A jury consisting of Professor P.W. de Leeuw, Dr C. Gaillard and Dr W. Hart selected the following papers:

**BEST ORIGINAL ARTICLE**

Candida-specific interferon-γ deficiency and toll-like receptor polymorphisms in patients with chronic mucocutaneous candidiasis


**BEST CASE REPORT**

Rituximab in the treatment of relapsing idiopathic thrombocytopenic purpura


**BEST REVIEW**

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