MISSION STATEMENT

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 speciality training and medical education, book reviews and correspondence.

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NOVEMBER 2003, VOL. 61, NO. 11
Mapping antibiotic use and resistance in the Netherlands: SWAB and NethMap

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ABSTRACT

The worldwide emergence of antimicrobial resistance has elicited responses from national and international organisations, including the World Health Organisation and the European Union. In the Netherlands, the non-profit foundation SWAB was jointly started by several professional medical societies to coordinate the Dutch efforts in preventing and reversing the trend of emerging resistance. SWAB publishes guidelines on the prudent use of antibiotics in this and other journals. The results of SWAB’s surveillance systems for antibiotic consumption and resistance were recently summarised in its NethMap 2003 document. Attention should now be focused on elucidating the major determinants of antibiotic use and resistance emergence, and designing effective intervention strategies to reverse the trend of resistance emergence.

The emergence of resistance to commonly used antimicrobial agents among medically important microorganisms poses a threat to the health of the public. Antimicrobial resistance generally increases the morbidity and mortality of patients suffering from infection and thereby increases the cost of healthcare delivery. Physicians aware of the emergence of resistance find themselves forced to change their antibiotic prescribing policies, not only in patients with proven infection, but also in patients with suspected infection. The economic impact of the emergence of antimicrobial resistance can, therefore, not be overstated. Resistance emergence drives the spiral of applying newer, ever more expensive, antimicrobial agents that have the built-in paradox of being less prone to existing resistance mechanisms and consequently pose a further selection pressure on the population of medically important microbial species in hospitals and the community.

Although it is clear that there is not a perfect correlation between in vitro resistance and therapeutic failure – the host’s innate and specific immunity systems play important roles here – there is now little doubt that resistance takes a heavy toll on society in terms of costs, morbidity and mortality. The emergence of methicillin-resistant Staphylococcus aureus (MRSA) in virtually all parts of the world is a good case study, a paradigm of the problem. Recent analyses of the impact of MRSA on clinical medicine has shown MRSA infection to be more difficult to cure, to be associated with higher levels of morbidity and mortality and to incur much greater costs for the healthcare system compared with infection due to methicillin susceptible strains of S. aureus (MSSA). It is also evident that in regions or countries where MRSA has emerged to clinically significant levels (>5-10%) the medical community has responded by switching their (empiric) antibiotic policies from the relatively inexpensive, safe and effective class of β-lactam antibiotics to much more expensive, less safe and potentially less effective classes of antimicrobial agents. In 2002, vancomycin-resistant clones of MRSA were detected reducing our choices further. Genetically it appears that resistance genes are acquired by those Staphylococcus aureus clones that are also successful as human pathogens. So where do we go from here?

In 1992 the Institute of Medicine of the United States of America published a landmark report regarding the...
emergence of infectious diseases and the threats these emerging diseases pose to the health in the United States. The report recommended that the World Health Assembly take the lead in promoting the development and implementation of a comprehensive global infectious diseases surveillance system. Indeed, the World Health Organisation (WHO) responded and formulated their global strategy for the containment of emerging and re-emerging infectious diseases shortly thereafter. The major elements of the WHO strategy were:
1. development and implementation of surveillance systems for antimicrobial agents;
2. promoting the surveillance of antimicrobial resistance among microbial pathogens;
3. upgrading of microbiology laboratories and microbiological expertise in many parts of the world;
4. to foster applied research into the determinants of emerging infections;
5. to emphasise prevention and control, rather than treatment of diseases.

In 2001 the WHO recognised that special efforts should be directed at containing the rapid emergence of resistance against antimicrobial agents and published their global strategy on the containment of antimicrobial resistance. This strategy largely concurs with the recommendations formulated at the same time by the European Union to address the menace of antimicrobial resistance where most other European countries have clinically seen a rapid increase in their resistance rates. NethMap 2003 reports relatively low levels of consumption and resistance over the years, but also signals trends in use and the emergence of resistance in some species against macrolides and fluoroquinolones.

As a consequence of the recommendations of the European Commission, all EU countries will need to produce such surveillance reports. These efforts are supported by national and European professional societies, and will in the coming years deliver a sharper image on the differences in antimicrobial use and resistance across Europe. As a continent, Europe seems to lead the way in managing the threat of antimicrobial resistance, where similar efforts in the USA, Japan and other parts of the world are fragmented or nonexistent. However, major tasks lie ahead for European countries as well. We have to know what the crucial determinants of antibiotic use are and explain the large differences in the current levels of antibiotic use among the EU member states. Also, for each relevant combination of antibiotic and microbial species we need to know what risk factors determine resistance emergence. Apart from better managing the use of antibiotics, the spread of resistant clones may make it necessary to upgrade our infection prevention efforts as well. Subsequently, we have to devise strategies to intervene with medical practices and deal with socioeconomic pressures that help drive the resistance spiral. NethMap will be monitoring our successes and failures.
The pathogenesis of systemic lupus erythematosus

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ABSTRACT

SLE is a complex, heterogeneous disease, the precise pathogenesis of which remains something of a mystery. In recent years our understanding has been advanced by the development of novel genetic and immunological techniques. Susceptibility to SLE has a genetic component and multiple putative genes are being investigated. The genes involved are likely to play a part in immune regulation. Central to the immune dysfunction seen in SLE is the presence of autoreactive B cells, which predominantly target nuclear antigens. In addition to evidence of aberrant B and T cell behaviour, lupus is associated with complement deficiencies, and abnormal cytokine function. A number of environmental triggers exist, and likely candidates include viral infection and exposure to UV light. Finally, evidence is accumulating that implicates apoptosis as a mechanism by which disease may be provoked and propagated.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease, characterised by the presence of autoantibodies. Virtually every organ or system can be involved, but commonly, SLE affects the skin, joints, haemopoietic system, kidneys, lungs and central nervous system. There is no simple answer to the question ‘what causes lupus?’ This heterogeneous disease is caused by the complex interaction of a variety of abnormalities which cause disease susceptibility, and/or provoke disease onset or exacerbation. At the core of this process is immune dysfunction, and the production of autoantibodies.

AUTOANTIBODIES

B lymphocytes from patients with SLE display a lack of self-tolerance, and an inappropriate overproduction of antibody. The presence of antinuclear autoantibodies (ANA) is the immunological hallmark of SLE. In clinical practice, ANA testing is often used as part of an initial investigative screen. A positive ANA is a sensitive test, found in 98% of patients with SLE, but the presence of anti-DNA antibodies is a much more specific finding. Anti-DNA antibodies are seen in approximately 60% of patients with SLE.

The precise role that anti-DNA antibodies play in lupus remains an area of great interest. Serial serum concentrations of these antibodies reflect disease activity in many patients, but not all. Instead of simply acting as a disease marker, it is now clear that some anti-DNA antibodies are, in some way, directly pathogenic. For example, studies have shown that injecting human hybridoma-derived anti-DNA antibodies into severe combined immunodeficiency (SCID) mice results, in some cases, in renal deposition of antibody with associated proteinuria. However, many questions remain unanswered. Since some patients have high anti-DNA antibody levels without overt disease, what are the critical structural features which determine pathogenicity? In addition, some patients have severe disease without detectable anti-DNA antibodies, so does this imply a different mechanism of disease?

In addition to anti-DNA antibodies, a variety of other autoantibodies are often detected. The antigens targeted may be associated with patient ethnicity (for example, increased levels of anti-Sm antibodies seen in Afro-Caribbean patients), or particular disease manifestations...
(for example, anti-Ro antibodies seen in association with a photosensitive rash). Finally, patients with lupus are often found to have positive antiphospholipid antibodies, with or without the related clinical syndrome.

**THE GENETICS OF SLE**

There is clearly a genetic component to disease susceptibility in SLE. Early evidence in support of this theory came from epidemiological studies of affected twins – monozygotic twins have a concordance rate of about 25%, compared with 2% in dizygotic pairs. More recently, genome wide screening has been used in an attempt to localise lupus susceptibility genes. This area is highly complex and there is considerable variation in the reported results. This variance may in part reflect methodology, but may also reflect the true diversity seen. The genes encoding HLA antigens would seem obvious potential targets, and in the Caucasian population, there does seem to be an association between HLA-DR2 and HLA-DR3. Interestingly, however, this association is not necessarily seen in other ethnic groups. A second area of potential linkage is mapped to the chromosome 1q region, which seems to stand out in affected sibling pair studies.

It seems likely that the genes implicated will have immune functions. Areas of interest include genes that encode proteins involved in antigen presentation (the HLA genes), apoptosis, the Fc receptor, B and T cell function, and the production of cytokines and complement.

**IMMUNE DYSFUNCTION AND SLE**

**B cells and T cells**

Central to the immune dysfunction seen in SLE is the existence of overactive B cells, which produce an abundance of autoantibody. The development and survival of these cells is dependent upon T-cell help. The propagation of self-directed B-cell clones may also be assisted by an inappropriate lack of T-cell suppression. B-cell activators, such as the protein B-lymphocyte stimulator (BLyS), appear to be upregulated in lupus, further encouraging B-cell survival.

Powerful new evidence for the strength of the role of B cells in disease development comes from a recent study of B-cell depletion therapy in patients with SLE, resistant to conventional therapies. Although only a small number of patients have been treated so far, results suggest a beneficial response in the majority. There is good data to show that immune cell signalling is abnormal in SLE. Stimulation of lupus B and T cells results in abnormally high free intracellular calcium concentrations and increased production of tyrosine phosphorylated proteins. This inappropriate response may account in part for the ‘overzealous’ behaviour of these cells.

**Complement**

Complement is involved in the clearance of immune complexes, and its function is somehow intertwined with the development of lupus. The association between genetic complement deficiencies and the development of lupus triggered early speculation about a possible role for complement in the aetiology of SLE. Furthermore, it was observed that in patients with SLE, complement consumption, with falling serum concentrations, often mirrors disease activity. With the increased interest in apoptosis (see below), the contribution of complement has become a hot topic once again. Defective clearance of apoptotic fragments may provide the link between complement dysfunction and SLE.

**Cytokines**

Cytokines are low-molecular-weight proteins which act as the chemical modulators of the immune system. It is easy to hypothesise, therefore, that they would seem a good potential site for dysfunction and, moreover, a convenient therapeutic target. Below, a selection of putative candidates are discussed.

IL-10 is secreted by T-helper cells, and stimulates B-cell proliferation and antibody production. There is an increasing body of research to suggest that this cytokine may be central to the overproduction of antibody seen in SLE. The serum concentration of IL-10 in lupus patients is significantly higher than that seen in normal controls. Stimulating lupus mononuclear cells with IL-10 causes significantly increased production of antibody. Moreover, SCID mice, injected with mononuclear cells from SLE patients and then treated with anti-IL-10 antibodies, display a marked reduction in the production of autoantibodies. Tumour necrosis factor α (TNFα) has also been investigated, and the evidence suggests that it may be protective against lupus. Linkage studies have demonstrated an association between low TNFα inducibility and an increased incidence of lupus nephritis, through the DR2 genotype. Conversely, DR3-positive patients have relatively high TNFα production, and are not predisposed to nephritis. The development of anti-TNFα drugs has provided a new angle on the hypothesis that blocking TNFα may be involved in the pathogenesis of SLE. The use of both of the commercially available anti-TNFα drugs, etanercept and infliximab, has been associated with the development of anti-DNA antibodies and, more rarely, a lupus-like syndrome.
However, other data suggest that the role of TNFα may not be so straightforward. For example, in a study looking at renal biopsies from patients with grade III and IV nephritis, approximately 50% of the samples exhibited TNFα deposition, suggesting a positive role in disease pathogenesis. Transforming growth factor β (TGFβ) is involved in the differentiation of CD8+ T cells into cells that downregulate the production of antibody. Ohtsuka et al. have looked at the function of TGFβ in lupus. Initial studies revealed that constitutive and active levels of TGF-β were decreased in these patients, when compared with controls. Moreover, treating lymphocytes collected from SLE patients with TGFβ resulted in the suppression of IgG production. Implying, therefore, that impaired secretion of TGFβ may in part account for the overproduction of antibody seen in lupus.

**APOPTOSIS**

In recent years, there has been growing interest in the role that apoptosis plays in the development of autoimmunity. Casicala-Rosen et al. demonstrated that the intracellular components that often make up the spectrum of target autoantigens in lupus cluster in blebs on the surface of apoptotic cells. This position enables them to be presented as antigen. Apoptosis is, however, a physiological process. Its part in the development of autoimmunity must, therefore, be dependent upon dysfunction elsewhere. In a recent editorial, Charles describes research findings that could account for this. Essentially, apoptotic fragments are usually rapidly cleared, minimising the production of an immune response. If, however, the rate of apoptosis overwhelms this function, or clearance is suboptimal, immunogenicity is increased.

Thus, apoptosis may provide a central pivot for disease production. Precipitating factors such as UV light, infections or drugs may cause increased apoptosis. Alternatively, they may induce dysfunctional clearance of apoptotic particles. This in turn results in increased exposure of the target antigens, and subsequent production of the corresponding autoantibodies. Conversely, reduced apoptosis has been implicated via a totally different mechanism. Evidence suggests that some T cells from patients with lupus overexpress the oncogene bcl-2, promoting cell survival by decreasing apoptosis. This could potentially allow autoreactive T cells to persist, propagating the autoimmune response.

**HORMONAL FACTORS**

Sex hormones play an immunomodulatory role in the development of autoimmune disease. SLE, in particular, predominantly affects women, with females commonly affected up to ten times more than males. Oestrogen is further implicated in the pathogenesis of lupus by the observation that SLE tends to affect women in the years between their menarche and menopause. Oestrogen can act as a potent disease stimulator in lupus-prone mice. In addition, there is evidence from mouse models that androgens may be protective against the development of autoimmunity. This observation has stimulated interest in the use of androgens as treatment for SLE. There are conflicting data about the risk pregnancy poses to women with SLE, but many clinicians worry about the precipitation of flares. There is also anxiety regarding the use of exogenous oestrogens, both in the oral contraceptive pill and hormone replacement therapy. The literature is hampered by a lack of prospective data, but in a recent review, Mok and colleagues concluded that the use of exogenous oestrogens does carry a risk of disease exacerbation. Moreover, in a group already at risk of thromboembolic disease, the use of hormonal treatments could be potentially harmful.

**ENVIRONMENTAL FACTORS**

**Viruses**

In the disease model that proposes SLE pathogenesis to be a combination of genetic susceptibility followed by exposure to an environmental trigger, viral infection provides a convenient putative target. Many possible culprits have been investigated. Epstein-Barr virus (EBV) is among the most popular candidates but even here, the evidence is patchy. There are also case reports and studies looking at a variety of other viruses, including cytomegalovirus, parvovirus B19 and the retroviruses. To date, however, no overwhelming evidence favouring a particular pathogen has emerged.

**Ultraviolet light**

Photosensitivity is a common presenting symptom of SLE. Ultraviolet (UV) light exposure causes rash and even systemic flare in susceptible individuals. Some patients are highly sensitive to this effect, and one case report describes exacerbation of cutaneous lupus following exposure to UV light emitted from a photocopier! Sontheimer reviewed proposed mechanisms for UV light induced lupus, and the hypothesis is as follows. As previously mentioned, anti-Ro antibodies are particularly associated with the development of a photosensitive rash. UV light exposure causes the release of proinflammatory cytokines and increases the rate of keratinocyte apoptosis. In combination, this causes exposure of autoantigens including Ro, and subsequent keratinocyte cytotoxicity.
CONCLUSION

Much progress is being made in increasing our knowledge of the aetio-pathogenesis of this complex disease. This understanding has brought with it the potential targeting of key molecules and the specific hope that this specificity will reduce the side effects associated with more general immunosuppression. Although the mortality associated with SLE has substantially reduced in the last decade, it remains a serious, potentially life-threatening condition, and careful long-term follow-up of patients with SLE remains paramount.

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23. Stolfi C, Bengtsson A, Klint C, Nived O, Sjoholm A, Truedsson L. Novel molecules and the reasonable hope that this specificity will reduce the side effects associated with more general immunosuppression. Although the mortality associated with SLE has substantially reduced in the last decade, it remains a serious, potentially life-threatening condition, and careful long-term follow-up of patients with SLE remains paramount.


Coagulopathy in prostate cancer

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ABSTRACT

Patients with metastatic hormone-refractory prostate carcinoma may have dramatic and life-threatening coagulation complications from their disease. We report here the case of a man with relapsing disseminated intravascular coagulation, and review the different coagulation disorders that may occur during prostatic carcinoma evolution. We focus mainly on disseminated intravascular coagulation (DIC), the most frequent coagulation complication. Other coagulopathies associated with prostate cancer are thrombocytopenic thrombotic purpura, thrombosis, Trousseau’s syndrome and acquired factor VIII inhibitor development.

INTRODUCTION

Prostate cancer is the most common cancer in men after skin malignancies. When metastatic, it becomes incurable and only palliative treatment can be offered. The most frequent metastatic sites are bone, then lymph nodes and the viscera. Patients with metastatic prostate cancer may experience complications due to widespread extension. Here we report the case of a man with relapsing disseminated intravascular coagulation, then review the different coagulation disorders possibly occurring in prostatic carcinoma. Their clinical presentations vary from haemorrhage to thrombotic manifestations. We will successively describe disseminated intravascular coagulation, thrombocytopenic thrombotic purpura, thrombosis, Trousseau’s syndrome and acquired factor VIII inhibitor occurrence.

CASE REPORT

A 61-year-old man with hormone-refractory prostate cancer and bone metastases was admitted with extensive chest-wall ecchymoses, bleeding at venipuncture sites, gingival haemorrhage and epistaxis. The platelet count was 54,000/ml (normal 130,000 to 400,000) and fibrinogen 0.09 g/l (normal 2 to 5). Clotting times were prolonged: prothrombin rate 20% (normal 60 to 100) and activated partial thromboplastin time 60 sec (normal 28 to 38), consistent with the diagnosis of acute disseminated intravascular coagulation (DIC). D-dimer test was positive (D-dimer >500 ng/ml) and soluble fibrin monomers were detected in blood. Prostate specific antigen (PSA) rate was 269 g/l (normal 0 to 4). The patient was treated with intravenous high-dose diethylstilbestrol diphosphate (Fosfestrol®), 1 g a day for five days. He was also transfused with packed red blood cells, platelets and fibrinogen. Within two weeks of treatment, the platelet count had increased to 96,000, and the fibrinogen count and clotting times had returned to normal. Subsequent oral oestrogen therapy was administered between intravenous (IV) courses. After three cycles of diethylstilbestrol diphosphate every three weeks, an attempt was made to interrupt the treatment but the patient was readmitted a few days later with biological findings of DIC. The platelet count was down to 96,000, and the fibrinogen count and clotting times had returned to normal. Subsequent oral oestrogen therapy was administered between intravenous (IV) courses. After three cycles of diethylstilbestrol diphosphate every three weeks, an attempt was made to interrupt the treatment but the patient was readmitted a few days later with biological findings of DIC. The platelet count was down to 19,000/ml, fibrinogen 1.1 g/l, and activated partial thromboplastin time was prolonged to 54 sec. There was no bleeding. Intravenous high-dose diethylstilbestrol diphosphate and heparin therapy were started. A positive response to the treatment was observed, then the patient remained well for several weeks. He was further re-admitted with ecchymoses and gingival haemorrhages. Laboratory analyses at entry showed low platelets.
prolonged prothrombin time (PT) and partial thromboplastin time (PTT), as well as decreased fibrinogen level. A new cycle of diethylstilbestrol diphosphate was started but the patient died from cerebromeningeal bleeding two days after admission. Clinical history and biological results are summarised in table 1.

**DISSEMINATED INTRAVASCULAR COAGULATION**

Disseminated intravascular coagulation represents the result of a widespread activation of coagulation pathways. Different clinical conditions, including solid tumours and haematological cancers, are associated with DIC. Other causes include infectious diseases (gram-negative sepsis), severe trauma, obstetric disorders, vascular diseases, reaction to toxins and immunological disorders. DIC is the most frequent coagulation complication in prostate cancer. The first reports published in the 1950s were presented as cases of apparent primary fibrinolysis. But medical observations by Rapaport in 1959 and Straub in 1967 recognised them as DIC with secondary fibrinolysis.

**Incidence**

DIC incidence in prostate cancer was historically found to be close to 25%. More recently, Ruffion reported this rate to be 13 to 30%, but clinical signs of DIC are actually found in only 0.4 to 1.65% of patients with prostate cancer. Prostate adenocarcinoma is the second solid malignancy, after gastric or pancreatic cancer, responsible for inducing DIC. In prostate cancer, the incidence of DIC is dependent on the tumour stage, and it is enhanced in metastatic hormone-refractory disease. Some authors have even proposed using coagulation indices as tumour markers in prostate cancer.

**Pathophysiology**

The pathogenesis of DIC proceeds from the simultaneous occurrence of systemic fibrin formation resulting from an increased generation of thrombin, impaired physiological anticoagulation mechanisms (low level of antithrombin III (ATIII) impaired function of the protein-C system, insufficient TPFI (tissue factor-pathway inhibitor)) and inadequate fibrinolysis. The combination of increased formation and impaired removal of fibrin results in thrombotic occlusion of small and midsize vessels. At the same time, there is a consumption of platelets and coagulation proteins at the site thrombosis, leading to possible bleeding. The difference between chronic (laboratory findings) and acute (severe clinical manifestations) DIC depends on the balance of intravascular clotting and on the platelet and clotting factor depletion. In chronic DIC, there is a slow generation of thrombin and a mild decrease in platelets and coagulation factors. In acute DIC, there is a massive generation of thromboplastic material, as well as a consumption of haemostatic elements. Compensatory mechanisms are not sufficient to restore coagulation proteins and platelets. This worsening could be explained by sepsis, radiation or chemotherapy, but it is also related to disease evolution. It may also be spontaneous.

The actual mechanism of this coagulopathy occurring in

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<td><strong>Clinical and laboratory data of patients with prostate cancer and relapsing DIC</strong></td>
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<td><strong>Physical examination</strong></td>
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<td><strong>Treatment</strong></td>
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PT = prothrombin time, APT = activated partial thromboplastin time, Diethylstilbestrol diphosphate = Fosfestrol®, 1g/d 5d.
cancer patients is not clear. A number of studies indicate that different procoagulant substances such as tissue factor (TF) expressed at the surface of tumour cells and a cancer procoagulant (CP) may be involved.14,15 Elsewhere, some authors have demonstrated that prostate tumour cells are rich in thromboplastin.16 Several proinflammatory cytokines, such as interleukin-6 and tumour necrosis factor, are supposed to be involved in DIC.17,18

**Diagnosis**

The diagnosis of DIC combines the following three features: any disease known to be associated with DIC, clinical manifestations, and a combination of laboratory tests. In 2002, Levi et al. proposed a scoring system using a five-step diagnostic algorithm to facilitate DIC diagnosis. This score can be obtained from routinely available laboratory tests (platelet count, fibrin-related markers such as fibrin(ogen)-degradation products (FDPs) and D-dimer, prothrombin time and fibrinogen level).19 The platelet count is typically decreased in DIC with often less than 100,000 platelet per cubic millimetre (normal count between 150,000 and 450,000). Prolongation of clotting times, such as prothrombin time and activated partial thromboplastin time, is found in 70% and 50% of patients, respectively.20 Fibrinogen concentration is low in only 50% of the patients, and it is usually associated with severe cases of DIC.14 A normal plasma fibrinogen level can be seen, particularly when the concentration prior to DIC was elevated due to neoplasia or sepsis.21 Fibrinolytic activation is documented by various tests. FDPs are increased in 85 to 100% of the patients with DIC. They reflect both fibrin and fibrinogen degradation and are only representative of the presence of plasmin. Circulating soluble fibrin monomers can be detected but, like FDP, are not specific for DIC. D-dimer assay by the ELISA method is more reliable for detecting DIC because it reflects fibrin (and not fibrinogen) degradation. In some situations, other laboratory tests are required. Evidence of procoagulant activity is demonstrated by elevated levels of prothrombin fragment 1 + 2 and fibrinopeptide A. Plasma levels of coagulation inhibitors such as ATIII and protein C are found to be decreased.12 These abnormalities are not specific but characteristic of DIC.12 They have been used to predict fatal outcome. Other biological abnormalities, such as the presence of schizocytes, can be seen but are not essential or specific to the diagnosis of DIC.14 They have been used to predict fatal outcome. Other biological abnormalities, such as the presence of schizocytes, can be seen but are not essential or specific to the diagnosis of DIC.14 They have been used to predict fatal outcome.

Other differential diagnosis is made on the absence of elevated D-dimers and the normal platelet and ATIII levels in primary fibrinolysis. The differential diagnosis with thrombotic thrombocytopenia purpura (TTP) is made on the normality of coagulation times in TTP.

**Clinical presentation**

Clinical features of DIC may vary from bleeding to thrombosis, or involve both. Schematically, four clinical situations can be discriminated.41

1. The patient is asymptomatic and chronic DIC is diagnosed by laboratory tests.
2. The patient presents with a thrombotic episode which is a manifestation of DIC (Trousseau's syndrome).22

Thrombosis presentation is not the commonest clinical feature of DIC but is often found at patient autopsy.59

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**Table 2**

*Clinical and biological findings in different coagulopathies*

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<tr>
<th>CLINICAL FINDINGS</th>
<th>LABORATORY FINDINGS</th>
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<tr>
<td>DIC</td>
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<td>Haemorrhages and/or thrombosis</td>
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<td>Thrombopenia</td>
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<td></td>
<td>PT normal</td>
</tr>
<tr>
<td></td>
<td>Fibrinogen normal</td>
</tr>
<tr>
<td>Anti-FVIII</td>
<td>Haemorrhages</td>
</tr>
<tr>
<td></td>
<td>↑ APT</td>
</tr>
<tr>
<td></td>
<td>↓ FVIII</td>
</tr>
<tr>
<td>TTP</td>
<td>Haemorrhages</td>
</tr>
<tr>
<td></td>
<td>Fever</td>
</tr>
<tr>
<td></td>
<td>Renal failure</td>
</tr>
<tr>
<td></td>
<td>Neurological abnormalities</td>
</tr>
<tr>
<td></td>
<td>Thrombopenia</td>
</tr>
<tr>
<td></td>
<td>Normal D-dimer test</td>
</tr>
<tr>
<td></td>
<td>Elevated ADAMTS13 activity</td>
</tr>
</tbody>
</table>

DIC = disseminated intravascular coagulation, PT = prothrombin time, APT = activated partial thromboplastin time, TTP = thrombotic thrombocytopenia purpura, LDH = lactate dehydrogenase.

---


**N O V E M B E R  2 0 0 3,  V O L.  6 1,  N O.  1 1**

349
3. Perioperative bleeding or minor bleeding (confined to the tumour area) occurs and patient presents a biological pattern of DIC.26,34
4. Acute, severe DIC with life-threatening haemorrhage can be observed. In this situation, hypovolaemia, hypotension and shock are probably related to cytokine production.35 Intravascular coagulation can then contribute to organ failure by compromising the blood supply. Renal failure or dysfunction of the pulmonary or central nervous system may also occur in patients with acute DIC.

Published cases often describe bleeding episodes corresponding to clinical situation 3. Moderate haemorrhage is generally consecutive to manipulations of prostate tumour tissue, at either the metastatic or primary site, or may also be spontaneous.4 Haematuria after prostate biopsy is the most frequent revealing sign of DIC in these cases.26-27 Perioperative bleeding in the course of decompressive laminectomy has also been reported.18 Unusually, gastrointestinal bleeding is the first manifestation of DIC.29 Impaired warfarin dose adjustment as an early manifestation of prostate cancer can be the first sign of chronic DIC.30

Therapeutic options
The dogma in DIC management is to first treat the underlying disorder.9 When laboratory signs of DIC are predominant or when bleeding is moderate (see above, clinical situations 1 and 3), initial treatment is directed specifically to the prostate carcinoma. But because of its occurrence in the course of metastatic hormone-refractory disease, causal DIC treatment is often difficult. The few specific therapeutic options published are hormonal manipulations (oestrogens, ketoconazole, orchiectomy), chemotherapy and radiopharmaceutical treatments.

Hormonal treatment is the basis of advanced metastatic prostate cancer treatment. In the hormone-refractory stage, third-line hormone treatment with oestrogens (diethylstilbestrol (DES) and diethylstilbestrol phosphate) has been proposed.34 Diethylstilbestrol phosphate has been found to be active in DIC related to prostate cancer35,36 but also to exacerbate signs.14 Hormone treatment is generally associated with antiaggregation treatment. In 1987, Lowe successfully used ketoconazole as an antiantiinogen in one case of DIC due to metastatic prostate cancer.15 Epsilon aminocaproic acid has also been shown to be active in some situations, though in association with high-dose intravenous DES,27 the drug is theoretically contraindicated because of the thrombotic risk, and not largely used. Chemotherapy has induced some results, particularly mitoxantrone24 but also more recently docetaxel and cisplatin.25 Radiopharmaceutical treatment is controversial: two publications have reported two patient deaths related to strontium-89 therapy.38,39 However, in 2000, Ruffion et al. described the case of a 61-year-old man with symptomatic DIC due to metastatic prostate carcinoma that could be controlled by treatment with samarium 153.8

Anticoagulant and particularly heparin treatment is still debated in the management of DIC. Few, small, nonrandomised studies have shown a benefit and no increase in bleeding with heparin in patients with DIC.40-41 No studies have been specifically conducted in patients with cancer, except in Trousseau’s syndrome.49 Continuous infusions of low-dose heparin are recommended (500-500 U/h).15 A treatment strategy involving antithrombin III (ATIII) replacement or protein C concentrates has also been used, but only in small nonrandomised studies.43,45 Most of these studies were performed in patients with sepsis or septic shock and in obstetrical circumstances.46 Some authors have used direct thrombin inhibitors (i.e. independent from ATIII) such as recombinant hirudin (r-hirudin) in haematological malignancies.48 But the clinical benefit of these treatments has not been clearly established.

Replacement treatment with blood components is determined by the importance of bleeding, the platelet count or coagulation factor levels. There is no evidence of prophylactic administration of platelets or plasma in patients with DIC.10 Some authors advocate replacing platelets when their count is below 50 x 10^9/l if the patient is bleeding or if an invasive procedure is needed.20 In case of important and life-threatening bleeding, fresh frozen plasma can be used. Because fresh frozen plasma contains more fibrinogen than cryoprecipitates, and cryoprecipitates are possibly contaminated with traces of procoagulation factors which could worsen the phenomenon, FFP should be given primarily.12

THROMBOCYTOPENIC THROMBOTIC PURPURA
Thrombocytopenic thrombotic purpura (TTP) is an acquired or congenital thrombotic microangiopathy, classically characterised by a pentad of signs: thrombocytopenia, microangiopathic haemolytic anaemia, neurological abnormalities, renal failure and fever.49 These abnormalities are the consequence of widespread microvascular thrombi, consisting in platelet aggregates with large amounts of von Willebrand factor and little or no fibrin (contrary to DIC),49 Its causal factor is a severe deficiency of von Willebrand factor–cleaving protease (1-4) known as ADAMTS13.50 Because of this deficiency, a large number of multimers of von Willebrand factor accumulate in the flowing blood, inducing platelet aggregation. TTP is sparsely described in prostate carcinoma.19 Other associated
diseases are metastatic malignancies, chronic inflammation, liver cirrhosis and systemic lupus erythematosus.\textsuperscript{54} Thrombocytopenic thrombotic purpura may also be induced by chemotherapy, particularly mitomycin C and cytotoxic associations such as bleomycin-cisplatin.\textsuperscript{49} Laboratory evaluations most commonly demonstrate haemolytic anaemia with the presence of schistocytes (fragmented erythrocytes), thrombocytopenia, elevated serum levels of lactate dehydrogenase and normal coagulation parameters. In acute episodes of thrombotic thrombocytopenic purpura, ADAMTS13 activity is found low in citrated plasma, and antibodies to ADAMTS13 may be detected.\textsuperscript{50} TTP is discriminated from DIC by pathogenesis and biological findings. It differs from haemolytic uraemic syndrome (HUS) by the presence of neurological or renal abnormalities (neurological traditionally predominant in TTP, renal in HUS), by ADAMTS13 level (normal in HUS).\textsuperscript{51} TTP is also differentiated from tumour microangiopathic haemolytic anaemia. The essential treatment for acquired TTP with ADAMTS13 deficiency consists of plasma exchange (plasmapheresis + infusion of fresh-frozen plasma or cryosupernatant). Additional treatment involving immune suppression by glucocorticoids, vincristine or splenectomy is considered in patients with high titres of inhibitor not responding to glucocorticoids, vincristine or splenectomy. In acute episodes of thrombotic thrombocytopenic purpura, ADAMTS13 activity is found low in citrated plasma, and antibodies to ADAMTS13 may be detected.\textsuperscript{50} TTP is discriminated from DIC by pathogenesis and biological findings. It differs from haemolytic uraemic syndrome (HUS) by the presence of neurological or renal abnormalities (neurological traditionally predominant in TTP, renal in HUS), by ADAMTS13 level (normal in HUS).\textsuperscript{51} TTP is also differentiated from tumour microangiopathic haemolytic anaemia. The essential treatment for acquired TTP with ADAMTS13 deficiency consists of plasma exchange (plasmapheresis + infusion of fresh-frozen plasma or cryosupernatant). Additional treatment involving immune suppression by glucocorticoids, vincristine or splenectomy is considered in patients with high titres of inhibitor not responding to plasma exchange. Platelet transfusions may exacerbate intravascular thrombosis and must be restricted to life-threatening haemorrhage.\textsuperscript{49}

THROMBOSIS

Activation of coagulation and predisposition to thrombosis is classically associated with most cancers, particularly prostatic carcinoma. In 1967, a large study of patients with advanced prostatic carcinoma showed that the incidence of thromboembolic disease was 2 to 3\% in early-stage disease and 8 to 9\% in more advanced forms.\textsuperscript{54} This hypercoagulability status results from several factors.\textsuperscript{55} Firstly, there are specific tumour properties including direct or indirect induction of thrombin generation either by cytokines or by tumour cell procoagulants (tissue factor (TF) and cancer procoagulant (CP)). Secondly, there are nonspecific factors, such as inflammatory state, tissue damage from tumour burden or necrosis leading to the activation of systemic coagulation. This clotting predisposition may have clinical expression or may remain latent with blood coagulation abnormalities. Several biological parameters are known to be a sign of hypercoagulability but none are specific. The most commonly seen are markers of platelet activation and thrombocytosis, markers of coagulation cascade activation (elevated levels of fibrinogen, modifications of prothrombin time and partial thromboplastin time), and suppression of fibrinolytic activity. All these phenomena lead to the activation of coagulation (elevation of fibrinopeptide A (FPA), presence of D-dimer (DD) and fibrinogen degradation products (FDP)). Some studies involving only few patients have shown that decreased ATIII levels may be associated with thromboembolic complications in patients with prostate cancer.\textsuperscript{57,58} Tumour procoagulant activity is principally reflected by two factors, namely cancer procoagulant (CP) and tissue factor (TF). Their role in the activation of coagulation is well known, but their plasma level is not well correlated with clinical thromboembolic disease.\textsuperscript{57} Another aspect of hypercoagulability is the inflammatory response. It has been studied in cancer patients, and studies have demonstrated the thrombotic role of two proinflammatory cytokines: tumour necrosis factor (TNF) and interleukin-I (IL 1). However, the role of these laboratory abnormalities is unclear. If many cancer patients have markers of coagulation activation, few will ultimately develop thrombosis. In 2002, Kohli showed an increase in coagulation markers (D-dimers, prothrombin fragment 1+2 (F1+2)) in 30 patients with advanced prostate cancer, as compared with age-matched control patients.\textsuperscript{59} Unfortunately, the clinical significance of these findings was not studied in these patients. In addition to these biological factors, extrinsic factors such as surgery, radiotherapy and chemotherapy can increase hypercoagulability characteristics. Besides, cytotoxic treatments and, particularly in prostatic carcinoma, hormone treatments can also induce hypercoagulability and be a source of thrombosis. Antiandrogenic compounds and oestrogens are known to enhance the thromboembolic risk. Oestrogens have been shown to decrease ATIII levels in DES-treated prostate carcinoma patients.\textsuperscript{57} Within more advanced-stage prostate cancer patients, the risk of thromboembolic disease is, however, heightened by the decreased mobility due to bone metastases and other comorbidities. In conclusion, cancer is associated with a complex multifatorial hypercoagulation status. No biological marker is actually sufficient for predicting thromboembolic accidents and identifying patients who may benefit from low-molecular-weight heparin prophylaxis. Curative initial treatment of thrombotic events is based on heparin.\textsuperscript{60} Low-molecular-weight heparins, which have been shown to be as effective and safe as unfractionated heparin, are ideal for outpatient management.\textsuperscript{65-64} Oral anticoagulant treatment is then ideal for long-term management. Theoretically, the treatment of venous thromboembolic events lasts six months but it should be continued indefinitely in patients with residual disease.\textsuperscript{65} The clinical management of long-term oral anticoagulant treatment in cancer patients is difficult because patients often require surgical procedures, have a therapy-related decrease in platelets and frequent therapy-related inter-
actions with the metabolism of vitamin K antagonists. Haemorrhagic complications are estimated at 2 to 3% annual risk.  

Trousseau’s syndrome is an association of migratory superficial phlebitis and underlying malignancy. It is now known to be a manifestation of chronic DIC.  

First described by Trousseau (1801-1867) in 1865, it is the condition most frequently associated with pancreatic and gastric carcinoma. It has also been reported in metastatic prostate carcinoma. The therapeutic attitude in Trousseau’s syndrome is similar to that of ‘classic’ DIC requiring the treatment of the underlying disorder. The use of intravenous heparin is always recommended when the tumour cannot be controlled. Oral treatment with anticoagulants such as warfarin is not adequate.

ACQUIRED FACTOR VIII INHIBITOR

Apart from DIC, bleeding manifestations in prostate carcinoma could result from the presence of acquired factor VIII inhibitor. This acquired inhibitory activity against clotting factor VIII is rare in prostate cancer and not clearly explained. In 2001, Sallah et al. reviewed all publications addressing acquired factor VIII inhibitor between 1974 and 2000 (41 patients) and found five cases (12%) associated with prostate cancer. The onset of this coagulopathy is often associated with a progressive disease. The diagnosis is generally made in the presence of unexplained bleeding. Laboratory tests show prolonged partial thromboplastin and kaolin coagulation times. Prothrombin time, platelet count and fibrinogen level are normal, therefore excluding a DIC. Diagnosis is confirmed by a decrease in factor VIII clotting activity and by the presence of FVIII inhibitors. Antibody titre is not directly related to bleeding complications and some patients are known to have had fatal bleeding with low-titre inhibitors.

Therapeutic options include factor replacement, immunosuppressive drugs and/or plasmapheresis. Treatment of the underlying malignancy is also required. Good therapeutic responses could be achieved with immunosuppressive drugs such as steroids, cyclophosphamide, cyclosporine or azathioprine. Best responses are obtained in patients with low-titre antibody. Human or porcine FVIII are indicated to treat haemorrhage and control acute episodes. When bleeding is persistent, additional use of prothrombin-complex concentrates that bypass the inhibitor (FVIII Inhibitor Bypassing Activity, FEIBA) or recombinant factor VIIa (Novoseven) is indicated. More recently, some authors have used rituximab in association with cytotoxic therapy in the management of patients with active bleeding and/or high-titre FVIII inhibitors.

CONCLUSION

Coagulation disorders are frequently associated with disseminated prostate cancer and should be known to urologists and oncologists because they may compromise short-term prognosis and influence therapeutic strategies. Disseminated intravascular coagulation is the most frequently reported disorder but, in spite of its long-time recognition, its treatment remains controversial.

REFERENCES

ABSTRACT

Background: This article presents cost-effectiveness analyses of the major diabetes interventions as formulated in the revised Dutch guidelines for diabetes type 2 patients in primary and secondary care. The analyses consider two types of care: diabetes control and the treatment of complications, each at current care level and according to the guidelines.

Methods: A validated probabilistic diabetes model describes diabetes and its complications over a lifetime in the Dutch population, computing quality-adjusted life years and medical costs. Effectiveness data and costs of diabetes interventions are from observational current care studies and intensive care experiments. Lifetime consequences of in total sixteen intervention mixes are compared with a baseline glycaemic control of 10% HbA1c.

Results: The interventions may reduce the cumulative incidence of blindness, lower-extremity amputation, and end-stage renal disease by >70% in primary care and >60% in secondary care. All primary care guidelines together add 0.8 quality-adjusted life years per lifetime.

Conclusion: In case of few resources, treating complications according to guidelines yields the most health benefits. Current care of diabetes complications is inefficient. If there are sufficient resources, countries may implement all guidelines, also on diabetes control, and improve efficiency in diabetes care.

INTRODUCTION

Ageing, lifestyle changes and improved case finding will increase the number of diabetes type 2 patients in most societies in the near future. In the Dutch population, diabetes led to a loss of 87,000 disability-adjusted life years in the year 1996, ranking 10th of all diseases. Diabetes contributes to the occurrence of cardiovascular disease, loss of vision and blindness, kidney failure, disorders of peripheral circulation and loss of sensitivity and pain in the legs, both leading to lower extremity ulcers and amputation. It is the largest cause of blindness in developed countries. About 15% of the dialysis patients in the Netherlands have diabetic nephropathy. In the United States, probably due to less diabetes control, this is 30%. Lower extremity amputation (LEA) is about 15 times more frequent among diabetes patients than in the general population. Healthcare costs related to diabetes and its complications are high in affluent societies and accounted for 2.5% of medical expenditures in the Netherlands in 1996. Cost-effectiveness analyses of diabetes guidelines are relevant for clinical and health policy reasons. Long-term clinical follow-up studies have demonstrated that intensive control of blood glucose is effective in reducing the risk of severe diabetes complications. Health economic studies have shown that intensive treatment might lead to lower healthcare costs, especially through fewer institutional episodes. Such studies typically report the costs and effects of an intervention given an existing level of control and treatment and hence are context-specific. It is in the interest of health policymakers to have more general...
information on allocation options in diabetes care given the various prevention and treatment options for complications.9,10 The premise of such analyses is that, for any given level of resources available, it is desirable to maximise the total aggregate health benefits.11-13 A comparison of health effects and costs of optional intervention mixes against a baseline care level facilitates priority setting at varying resource levels. The efficiency of current interventions may be considered.13 In this article a low diabetes control level of 10% glycosylated haemoglobin (HbA1c) is taken as baseline.

In the Dutch setting, primary care physicians are the gatekeepers for secondary care facilities. About 80% of type 2 diabetes patients are treated in primary care and are referred only temporarily for secondary care consultation, for example for eye screening.14 Specialists in ambulatory secondary settings only treat the more difficult cases. Here we present analyses for combinations of various intervention mixes as formulated in the Dutch guidelines for diabetes type 2 care15-17 and report on the allocation options at different resource levels. We consider two sets of intervention mixes for diabetes patients: one for those in primary care and one for those in secondary care.

**METHODS**

We estimate health effects and medical costs of current care and care according to guidelines in the two groups compared with a baseline setting. We collected data on current care and used data on two experimental guideline settings.9,10 We first summarise the application of the disease history model for diabetes. Then, we describe the computations to arrive at validated baseline estimates. Last, to obtain comparable cost-effectiveness results, we give the details on the input values for the effectiveness and costs for the two sets of, in total, eight possible intervention mixes for each set.

**Multi-state disease model**

We modified a probabilistic Markov model to describe the Dutch diabetes situation.20 It describes the disease history of type 2 diabetes and calculates quality-adjusted life years (QALYs) lived with diabetes and its complications, as well as lifetime medical costs. We refer to the original publication for detailed description. Figure 1 gives an overview of the model. It computes the occurrence of the mild and severe long-term diabetic complications and the excess mortality due to diabetes. The model distinguishes five health states for retinopathy, four for nephropathy and three for neuropathy. Patients may progress from states without specific complications, through less severe intermediate stages, towards three severe diabetes complications, leading to severe vision loss (<20/100), kidney failure or lower extremity amputation. The intermediate retinopathy states are background retinopathy, macula oedema and proliferative retinopathy. For nephropathy these are microalbuminuria and gross proteinuria, leading to end-stage renal disease (ESRD). The neuropathic complications are leg and foot ulcers and LEA, as results from ‘diabetic foot’. The model describes cohorts of diagnosed diabetes patients. They enter the model one by one through stratified random sampling until a stabilisation of results occurs. It accounts

![Figure 1](image-url)

**Figure 1**

*Overview of groups of disease states in diabetes model and action effects of intervention mixes*

The actual number of possible disease states is higher; see text.20
for their age and sex distributions and the distribution of their HbA1c levels (table 1). The complication probabilities are specific for age, gender and diabetes duration. There are two independent mortality risks. One accounts for diabetes-specific mortality and the other for the excess mortality. The latter includes the excess cardiovascular mortality risk. Figure 1 indicates that progression towards severe states depends on both the level of diabetes control and the level of specific treatment during the less severe intermediate stages.

### Table 1

Model input values for diabetes control and preventive treatment of complications by patient group characteristics, effectiveness and annualised medical costs (1996 €)\(^{17-19,21,22}\)

<table>
<thead>
<tr>
<th>LEVEL OF CARE</th>
<th>Current primary care (P1.CC + P2.CC)</th>
<th>Primary guidelines care (P1.GC + P2.GC)</th>
<th>Current secondary care (S1.CC + S2.CC)</th>
<th>Secondary guidelines care (S1.GC + S2.GC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients in survey</td>
<td>1371</td>
<td>459</td>
<td>929</td>
<td>1029</td>
</tr>
<tr>
<td>Mean age (SD)</td>
<td>65.2 (11.7)</td>
<td>66.1 (12.5)</td>
<td>69.2 (11.5)</td>
<td>69.2 (11.5)</td>
</tr>
<tr>
<td>Gender distribution (% men)</td>
<td>49</td>
<td>39</td>
<td>43</td>
<td>41</td>
</tr>
<tr>
<td><strong>Diabetes control (P1 and S1)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Effectiveness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average HbA1c % (S.D.)</td>
<td>7.6 (1.5)</td>
<td>7.0 (1.3)</td>
<td>7.8 (1.5)</td>
<td>7.2 (1.3)</td>
</tr>
<tr>
<td>Proportion of patients &lt;7.0%</td>
<td>0.44</td>
<td>0.54</td>
<td>0.25</td>
<td>0.35</td>
</tr>
<tr>
<td>Proportion of patients &gt;8.5%</td>
<td>0.28</td>
<td>0.12</td>
<td>0.24</td>
<td>0.15</td>
</tr>
<tr>
<td>Proportion of insulin patients</td>
<td>0.04</td>
<td>0.16</td>
<td>0.74</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>Medical costs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visits to general practitioner</td>
<td>128</td>
<td>318</td>
<td>128</td>
<td>318</td>
</tr>
<tr>
<td>Visits to various diabetes specialists</td>
<td>144</td>
<td>120</td>
<td>212</td>
<td>298</td>
</tr>
<tr>
<td>Visits to diabetes nurses</td>
<td>63</td>
<td>218</td>
<td>109</td>
<td>218</td>
</tr>
<tr>
<td>Visits to paramedics</td>
<td>0</td>
<td>184</td>
<td>48</td>
<td>120</td>
</tr>
<tr>
<td>Oral drug, insulin; self-control</td>
<td>347</td>
<td>386</td>
<td>977</td>
<td>1937</td>
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<tr>
<td>Laboratory tests</td>
<td>40</td>
<td>187</td>
<td>40</td>
<td>271</td>
</tr>
<tr>
<td><strong>Treatment less severe complications (P2 and S2)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Effectiveness (probability reduction)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laser coagulation in ME, postponing blindness/low vision</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Laser coagulation in PDR, postponing blindness/low vision</td>
<td>0.08</td>
<td>0.015</td>
<td>0.08</td>
<td>0.015</td>
</tr>
<tr>
<td>ACE inhibitors in gross albuminuria</td>
<td>0.08</td>
<td>0.05</td>
<td>0.27</td>
<td>0.05</td>
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<tr>
<td>Foot clinic treatment neuropathy</td>
<td>0.17</td>
<td>0.05</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Medical costs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye screening visit</td>
<td>27</td>
<td>55</td>
<td>27</td>
<td>55</td>
</tr>
<tr>
<td>Laser coagulation + follow-up</td>
<td>272</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Visits diabetic foot clinic</td>
<td>20</td>
<td>58</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td><strong>Treatment severe complications</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Medical costs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blindness</td>
<td>1200</td>
<td>2550</td>
<td>660</td>
<td>3200</td>
</tr>
<tr>
<td>End-stage renal disease</td>
<td>46,700</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic foot ulcer</td>
<td>563</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEA event/amputation status</td>
<td>12,000/450</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Baseline estimates
We applied the disease model to compute a baseline situation (table 1). HbA<sub>1c</sub> indicates the level of diabetes control and it is directly related to the occurrence of complicating events later in life. We used this observed control and it is directly related to the occurrence of current care and care according to the revised guidelines. Effectiveness preventive treatment of complications (table 1) lists the input values for diabetes control and treatment of complications by patient group and by level of care. For instance, the single option P1.CC means diabetes control as currently given and there is no treatment of complications in primary care. In total, we analyse sixteen of those options of diabetes interventions (table 2 and 3).

Effectiveness diabetes control
Empirical data regarding the level of diabetes control in current and guideline settings (P1.CC, P1.GC, S1.CC and S1.GC) have been collected in three studies. The HbA<sub>1c</sub> figures for primary care patients (P1.CC and P1.GC) are based on a two-year follow-up of 459 patients in 22 primary care practices. Effectiveness figures for current secondary care patients are from a survey in ten general hospitals among 929 patients. Accounting for control effectiveness (versus trial efficacy) we entered the observed distributions of all HbA<sub>1c</sub> values into the probabilistic calculations instead of the observed means. Table 1 shows the HbA<sub>1c</sub> fractions for those values >8.5% and for those between 7.0 and 8.5%. It indicates, for example, that in all four groups more than 10% of the patients remain above the 8.5% HbA<sub>1c</sub> level. The relationship between HbA<sub>1c</sub>, level and progression to diabetic complications is estimated by a function reported earlier. It has been validated for the Netherlands and is based on the formula \((HbA_{1c}/10)^{\beta}\). The calculated fraction is the reduction of the transition probabilities towards each of the three complication categories. The \(\beta\)-coefficients are specific for each type of less severe complication. The function shows diminishing returns when lowering HbA<sub>1c</sub> level through more intensive diabetes control. The UKPDS study has confirmed the degree of diminishing returns. The guidelines for diabetes control aim at lower levels of HbA<sub>1c</sub> and the guidelines for complications recommend frequent screening and preventive treatment though laser coagulation, ACE inhibitors and foot clinic visits.

Input data for two sets of intervention mixes
We collected data for the two types of intervention sets (diabetes control and treatment of complications) for each of the two patient groups (table 1). The difference between the primary and secondary care group is that in the latter diabetes control is more difficult and severe complications are more frequent. Both conditions are indications for a referral according to the guidelines. Both types of intervention are considered at two different levels of care i.e. current care and care according to the revised guidelines.

Effectiveness preventive treatment of complications
The effectiveness figures for the treatment of retinopathy and nephropathy are from experimental trials and have been reported before. In macula oedema, laser coagulation

Table 2
Lifetime cumulative incidence (%) of diabetes complications by intervention mix component

<table>
<thead>
<tr>
<th>TYPE OF COMPLICATION</th>
<th>INTERVENTION MIX COMPONENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BASELINE</td>
</tr>
<tr>
<td></td>
<td>P1.CC</td>
</tr>
<tr>
<td>Background retinopathy</td>
<td>73.6</td>
</tr>
<tr>
<td>Macular oedema</td>
<td>38.3</td>
</tr>
<tr>
<td>Proliferative retinopathy</td>
<td>8.7</td>
</tr>
<tr>
<td>Low vision/blindness</td>
<td>13.5</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>36.4</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>25.2</td>
</tr>
<tr>
<td>ESRD</td>
<td>5.6</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>19.7</td>
</tr>
<tr>
<td>Lower extremity amputation</td>
<td>7.7</td>
</tr>
</tbody>
</table>

P = primary care, S = secondary care, 1 = diabetes control, 2 = care of complications, CC = current care, GC = guideline care, ESRD = end-stage renal disease.

Table 3
QALYs lived and medical costs (1996 €) per average remaining diabetic lifetime for the two independent sets P and S of intervention mixes, ordered by QALYs lived

<table>
<thead>
<tr>
<th>INTERVENTION MIXES</th>
<th>NO. SINGLE SET MIXES</th>
<th>NO. COMBINED P AND S MIXES</th>
<th>MODEL OUTPUTS</th>
<th>COST-EFFECTIVENESS RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>QALYs LIVED</td>
<td>LIFETIME COSTS</td>
</tr>
<tr>
<td>0</td>
<td>Baseline care</td>
<td></td>
<td>9.294</td>
<td>2626</td>
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<tr>
<td>1</td>
<td>S2.CC</td>
<td></td>
<td>9.384</td>
<td>349</td>
</tr>
<tr>
<td>2</td>
<td>S1.CC</td>
<td></td>
<td>9.470</td>
<td>1403</td>
</tr>
<tr>
<td>3</td>
<td>S2.GC</td>
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<tr>
<td>4</td>
<td>S1.GC + S2.CC</td>
<td></td>
<td>9.445</td>
<td>2642</td>
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<tr>
<td>5</td>
<td>S1.CC + S2.CC</td>
<td></td>
<td>9.427</td>
<td>1384</td>
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<tr>
<td>6</td>
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<td></td>
<td>9.433</td>
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<tr>
<td>7</td>
<td>S1.GC</td>
<td></td>
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<td>8</td>
<td>S1.GC + S2.GC</td>
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<tr>
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<td>8648</td>
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<tr>
<td>18</td>
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<td></td>
<td>10.236</td>
<td>9665</td>
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<tr>
<td>25</td>
<td>P1.GC + P1.GC + S1.GC</td>
<td></td>
<td>10.249</td>
<td>10,937</td>
</tr>
</tbody>
</table>

Each set includes eight mutual exclusive mixes. Mixes in bold indicate one optimal expansion path. In the last column the CERs are relevant to this expansion path. Here, in each step, the preceding optimum mix is the reference intervention. QALYS = quality-adjusted life years, baseline care = exclusively treatment of severe complications (see costs in table 1), SD = standard deviation, CER = cost-effectiveness ratio (Euro/QALY), P = primary care, S = secondary care, 1 = diabetes control, 2 = care of complications, CC = current care, GC = guideline care.
slovene costs by intervention mix
We collected data regarding healthcare utilisation from the same three studies and did a large cross-sectional study of primary care patients. This study reports the actual health utilisation and costs from 29 general practices of 1371 primary care patients. Health utilisation estimates for current secondary care are from a hospital survey.21 The cost estimates for the implementation of guideline care are from two experimental studies applying intensive treatment protocols in primary and secondary care patients.8,9 Table 1 lists the cost input values for diabetes control and treatment for four categories of patients (P.CC, P.GC, S.CC, and S.GC). Medical costs of amputation, follow-up after amputation, end-stage renal disease and blindness are assumed the same in all four patient groups. The calculated lifetime cost estimates do not include the medical costs of nondiabetes-specific conditions. We provide more cost details in the report.7

RESULTS

We computed lifetime health effects and medical costs for the sixteen diabetes intervention mixes in the two sets. One set includes all possible mutual exclusive intervention mixes for primary care (P) and the other (S) includes all possible mutual exclusive mixes for secondary care. We first present the specific health effects for the eight single components of the intervention mixes (P1, P2, S1, S2) for current care and guideline care (CC and GC). Next, we present effects and costs of the eight single components and eight combined mixes for control and preventive treatment (P1 + P2 en S1 + S2). This leads to results for in total sixteen intervention mixes as listed in table 2.

Health effects
Table 2 shows the incidence of complications for patients under the four intervention mixes (P.CC, P.GC, S.CC, and S.GC). It compares the effects of each single component, i.e. diabetes control (P1 or S1) and preventive treatment of complications (P2 or S2) with the baseline estimates. The first column gives the results of the baseline scenario. Diabetes control reduces the incidence of all complications. Once less severe complications occur, preventive treatment reduces progression to severe complications. Some 74% of type 2 diabetes patients developed background retinopathy under the baseline scenario, whereas blindness occurs in 13.5%. Under current level of control, this is reduced by more than 75%. Implementation of control guidelines among primary care patients reduces the cumulative incidence of blindness by more than 90%, whereas ESRD falls by 67% from 5.6% to less than 0.5%. The cumulative incidence of diabetes-related amputations decreases from 7.7% in the baseline to 2.1% in the current primary care setting. Similar, less substantial declines take place among the more complex patients in ambulatory secondary care. Implementation of secondary care guidelines leads to a reduction of blindness by 29%, of ESRD by 62%, and of LEAs by about 27%.

Table 2 also shows that the incidence of these severe complications results in more patients with less severe complications in the case of blindness (P2.GC and S2.GC) and amputations (S2.GC). This leads to a relative increase in costs. Reductions due to specific single treatments of complications (not listed) are substantial, but lower. Patients in current care with higher initial HbA1c levels benefit more from guideline control than those with lower initial values of HbA1c.

Costs-effectiveness of diabetes interventions
Figure 2 and table 3 present the means of the computed QALYs lived and the discounted additional lifetime costs per average diabetes patient for the sixteen possible combinations of the four intervention mixes (P.CC, P.GC, S.CC, and S.GC). The standard deviations for the QALYs lived vary between 5.04 and 6.01 years and for the lifetime costs between € 3103 and € 8265. The calculated baseline life expectancy is 9.29 QALYs (SD=5.3). The SD value compares well with observed figures for the unadjusted life expectancy (CBS, 1992). The large SDs for lifetime costs are due to the large variation in remaining life years lived and the less frequent occurrence of the most costly complications. This reflects clinical reality in the treatment of older individual patients: given the high individual risks of dying from other causes, future health benefits and medical costs are uncertain at the individual level.

The higher costs of guideline control (table 1) and the treatment costs of complications are partially offset by reductions in the costs of severe complications, especially by savings on the care of severe renal and lower extremity
complications. All primary care guideline interventions together (P1.GC + P2.GC) show the highest health yield for a single intervention set: about 0.8 QALY per average lifetime. As a single intervention, eye screening and laser coagulation (not listed) fall within the same range of cost-effectiveness. The cost-effectiveness ratios for current treatment for renal and lower extremity complications (not listed), as single interventions, are much higher. Diabetes control in secondary care patients is still more costly per unit HbA1c reduction. This explains why primary control is more cost-effective than specialist control. As the current control level is already high in both primary and secondary care, even tightened control shows increasing costs and diminishing returns.

The two guideline intervention mixes for complications (P2.GC and S2.GC) are dominant compared with the current care of complications (P2.CC and S2.CC). Guideline treatment of complications (P2.GC and S2.GC) is cost-effective for three reasons: the intervention costs are low, the effects are immediate in a large majority of patients, and the indicated patient subgroup is relatively small. In diabetes control, annual costs are higher, health gains occur later in life, and many patients need to be treated to prevent relatively few, severe and costly complications. Therefore, current control is less cost-effective than preventive treatment of complications. Intensive control is even less cost-effective.

Table 3 and figure 2 indicate one possible optimal resource expansion option, namely how to prioritise implementation of efficient diabetes care starting from a baseline level. Here, one would start by choosing the most cost-effective option at the lowest budget needed, followed by the next cost-effective and so forth, until resources are exhausted. In table 3, only the relevant combinations of P and S are listed (columns three, numbers 17-25). Other combinations are possible but not relevant for the path. For the sets of mutual inclusive interventions (P and S) the order would be to start with the guidelines treatment for complications, next to add primary control, and lastly to implement intensive secondary control. The optimum expansion path for all combinations of all possible P and S mixes starts with S2.CC. This is the most efficient and least expensive option: in other words, it gives most savings, compared with baseline level (table 3). The specific implementation steps would be to improve this to S2.GC, add P2.GC, add P1.CC, improve this to P2.GC, and lastly to include the remaining S2.GC option. At mid-range budgets also other, single and combined, mixes are on other expansion frontiers, for example adding S1.CC after the implementation of P2.GC and S2.GC. S1.CC (figure 1) can be implemented
at much lower costs, but is three times less cost-efficient, at € 21,897 per QALY. At higher budgets, health effects and the absolute costs for secondary care patients are less influential due to the relatively small size of this group. Health gains in this group, although very inefficient (figure 2), need few additional euros per average lifetime. Many more expansion paths are possible if uncertainties such as standard deviations of health effects and lifetime costs are taken into account. In the uncertainty analysis all these paths are considered together; however this did not change the conclusions.29

DISCUSSION

Our analyses show that the diabetes care guidelines are cost-effective in reducing severe and expensive complications. This reconfirms the results of other studies.3,8 They also show that implementation of the guidelines for complications both in primary and secondary care reduces the current inefficiencies in diabetes care. In case of low available resources, a combination with moderate diabetes control (Pt.CC) is a good option. Also while including uncertainties, the mixes that include guideline treatment of complications continue to be a likely optimum choice. At high resource levels, all primary and secondary care guidelines are relevant. The interventions in secondary care are cost saving compared with baseline; those for primary control cost about € 6000 to € 7000 per QALY gained.

Cost-effectiveness methodology

The inclusion of a baseline scenario as a reference level is one way to operationalise the generalised cost-effectiveness analysis (CEA) approach of the WHO.11,13 Our baseline scenario represents the average low controlled diabetic still receiving care for severe complications. Estimates for this situation can be relatively well documented as the relationship between HbA1c blood values and the occurrence of complications is well established. However, the exact natural history of diabetes, when no treatment at all is given, remains unknown. The first advantage of our approach is the possibility to assess the relative efficiency of the current mix of care. For the Netherlands, data on the level of current diabetes care have recently become available.26 The present study shows that, due to undertreatment, current primary care of complications is inefficient as more costs due to severe complications can be prevented (table 3). In a direct, context-defined, comparison of current care and guidelines care this would show as cost savings such as those we demonstrated elsewhere for diabetes nephropathy.1 The comparison with a baseline level makes the information for health policymakers more complete and indicates the level of expenditures still needed.

The second advantage is the possibility to consistently compare intervention mixes for two (or more) different subpopulations at different available budgets after choosing the right denominator. The unit of analysis is the average cost per diabetic lifetime. Given the small numbers of patients, the provision of secondary care leads to low average lifetime costs for all diabetics, in spite of high individual costs and higher cost-effectiveness ratios. In case of a low budget, preventive treatment of these patients according to this analysis deserves priority. This is only one way to define the optimum benefit given a fixed health budget to spend for the diabetes population. QALYs and costs for both groups of patients in our analysis have the same weights and have the same denominator (the average diabetic lifetime). Different health policy criteria, such as equity considerations, might lead to different weights, for example priority to the more disabled.9 In this case, the policymaker might choose one of the less likely, nevertheless optimum, options.

There is an indirect interdependence between the health gain and costs due to diabetes control and due to the specific treatment of mild complications. Both reduce severe complications. In a sense, the diabetes health states act as communicating vessels. Better control leads to fewer patients needing preventive treatment of complications. Absence of diabetes control leads to more patients with complications. Treatment of complications in the absence of control leads, on average, to more health gain and higher costs. The disease history model accounts for this interdependence. Table 2 illustrates these results in both the single and combined scenarios.

The baseline estimates are difficult to validate. It might be possible to use a specific calendar as a reference situation, computing ‘backwards’.3,22 We did this and presented some historical evidence. Our baseline quality-adjusted life expectancy of 9.3 QALYs due to low diabetes control is probably an overestimation. At a mean 10% HbA1c level, there will be loss of health due to direct metabolic complications, leading to less QALYs and higher costs in the baseline scenario. This would lead to more favourable cost-effectiveness ratios for the intervention sets. Certainly within limits, it does not make an essential difference which baseline is chosen as long as its health effect values are substantially lower than the computed gains for the actual interventions.

Our main conclusions on the optimum mixes, however, are based on the relative values for health benefits and costs of the studied intervention mixes, starting with the optimum choice at the lowest budget level. This does not change for different baseline values, nor would the relative values for the interventions change. A comparison with interventions for other diseases to compute the net population benefit, however, would mean that the baseline values need redefining to include the characteristics of the other patient (or
population or high-risk) groups involved. Uncertainties in other model input values, such as those for discounting, utility weights or transition probabilities, do not change the set of relative values substantially either.

CONCLUSION

In case of low resource availability (<€ 300 per diabetes lifetime), none of the diabetes mixes is a relevant policy option. Highly likely optimal strategies in resource-poor countries are the implementation of guideline treatment of complications and primary diabetes control (P2.GC, S2.GC, and P2.CC). Our study shows the most likely cost-effective options. However, other allocation criteria will influence the decision-making.

In countries with high resources, priority should also be given to the guideline treatment of complications as current diabetes care shows inefficiencies. At a budget of over € 12,000 per diabetes lifetime, one can afford the implementation of all interventions, although at the individual level uncertainties are high.

The implementation results depend very much on the strategies followed.\textsuperscript{35} Simply distributing guidelines seldom leads to (cost)effective implementation.\textsuperscript{3536} Other constraints in a cost-effective implementation are an already high existing level of control and the lack of sufficient improvement in many diabetics. There are diminishing returns in intensive diabetes control. Further selection of high-risk subgroups, by age, sex, risk factor status and HbA\textsubscript{1c} level, may lead to the identification of more specific, targeted and cost-effective implementation strategies. For this, it will be necessary to conduct wider-scale and more targeted evaluations of impact and costs of different implementation practices of diabetes guidelines.

ACKNOWLEDGEMENTS

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NOTE

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REFERENCES

Chronic mucocutaneous candidiasis (CMC) is a group of disorders, characterised by persistent mucocutaneous infections with *Candida* species. The underlying defect of CMC has not been elucidated, but a defective cytokine response may be involved. Therefore, we investigated whether an imbalance between IFN-γ and IL-10 may play a role in this disorder.

We assessed the cytokine production in whole-blood cultures from CMC patients using *Candida albicans*, lipopolysaccharide and phytohaemagglutinin as stimuli. As the Toll-like receptors are important pattern recognition receptors for *Candida* species, we also investigated Toll-like receptor polymorphisms in these patients.

Patients with CMC had a significantly decreased IFN-γ production when whole blood was stimulated with *C. albicans* (232 ± 120 vs. 2279 ± 609 pg/ml, p<0.02). When stimulated with phytohaemagglutinin, the differences were not significant (3549 ± 1320 vs. 7631 ± 1790 pg/ml).

The *Candida*-stimulated production of IL-10 tended to be higher in CMC patients, whereas TNF and IL-1β production were similar in patients and controls. Stimulation with LPS showed no differences in cytokine production between patients and controls. Two out of seven patients had the TLR4 Asp299Gly polymorphism and none had the TLR2 Arg677Trp polymorphism.

These data support the hypothesis that deficient IFN-γ production is involved in the pathogenesis of CMC, whereas a role for genetic polymorphisms of Toll-like receptor 2 and 4 is not obvious in these patients.

**INTRODUCTION**

Chronic mucocutaneous candidiasis (CMC) is a group of disorders characterised by persistent mucocutaneous infections with *Candida* species. Several clinical variants of CMC have been described, some of which are associated with endocrinopathies or autoimmune diseases, such as hypothyroidism and hypoparathyroidism. Patients with CMC rarely develop disseminated or invasive candidiasis, suggesting a defect in the host defence limited to superficial candidal infections.

It is generally accepted that such defence mechanisms encompass macrophages, cytotoxic lymphocytes and natural killer (NK) cells. For activation of these cells, proinflammatory cytokines such as interferon (IFN)γ and tumour necrosis factor (TNF) are major mediators, whereas anti-inflammatory cytokines, such as interleukin (IL)-4 and IL-10, antagonise the cellular anticanidal defence. Production of these cytokines is initiated by recognition of the micro-organism by pattern recognition receptors, especially Toll-like receptors (TLR), on the cellular surface. TLR2 is the main receptor involved in induction of proinflammatory cytokines after stimulation with *Candida albicans*, while TLR4 mediates chemokine production. The balance between T helper (Th1) and Th2 cytokines is important in the initiation of the type of immune response. A Th1 cytokine response is associated with resistance to candidiasis, whereas a Th2 response results in susceptibility to infection.

It has been hypothesised that a defective Th1 response may be at least partially responsible for the persistence of...
Candida infection in CMC patients. To further test this hypothesis, we assessed the pro- and anti-inflammatory cytokine response in a whole blood culture model after stimulation with C. albicans, lipopolysaccharide (LPS) and phytohaemagglutinin (PHA) in patients with chronic mucocutaneous candidiasis. In addition, we investigated whether known polymorphisms in TLR2 or TLR4 genes, which are associated with impaired cytokine production, could be involved in the pathogenesis of CMC.

PATIENTS AND METHODS

Seven patients with CMC (three males and four females, aged from 8 to 55 years) were studied. For each patient, two healthy age- and sex-matched controls were included. During the study, the CMC patients did not suffer from other concurrent disorders or acute infections. After obtaining informed consent, blood samples were obtained from both patients and controls at the same time, using 2 ml glass tubes containing lithium heparin (Becton Dickinson, Franklin Lakes, NJ).

Ex vivo cytokine production

The whole blood was diluted 1:5 with RPMI 1640 Dutch Modification (ICN Biomedicals, Aurora, OH) in 24-well plates (Costar Corning, New York, NY). Phytohaemagglutinin (PHA; 10 μg/ml; Sigma Chemical Co., St Louis, MO), E. coli lipopolysaccharide (LPS; 1 ng/ml; Sigma) or heat-killed C. albicans (1 x 10^6 cfu/ml or 1 x 10^7 cfu/ml, heat-killed for 30 minutes at 100°C) were added. Each well contained a final volume of 1 ml. The samples were incubated for 24 or 48 hours at 37°C in 5% CO₂ atmosphere. Supernatants were collected after centrifugation and stored at -20°C until tested.

Circulating cytokine concentrations

For the analysis of circulating cytokine levels, the blood samples were centrifuged and the plasma was collected. The samples were stored at -20°C until analysis. The concentrations of TNF, IL-1β and IL-1Ra were measured by specific radioimmunoassay. Concentrations of IFNγ and IL-10 were measured by ELISA according to the guidelines of the manufacturer (CLB, Amsterdam, the Netherlands). Detection limits of the assay were IFNγ 2.5 to 200 pg/ml; IL-1β 0.04 to 1.25 ng/ml; IL-10 1.25 to 200 pg/ml; IL-1Ra 0.08 to 0.8 ng/ml; and TNF 0.02 to 1.0 ng/ml.

TLR2 and TLR4 polymorphisms

TLR2 and TLR4 polymorphisms were assessed in the CMC patients and in 200 healthy Dutch controls, participating in a health survey for recurrent venous thrombosis. Genomic DNA was isolated from blood by using the Puregene DNA isolation kit (710001, Gentra systems, BIOzym, the Netherlands). The DNA was stored at 4°C until the analysis. To determine the TLR4 genotype, the DNA was amplified with primers (forward primer: 5’ ATACCTAGACTACTACCTCATG 3’, reverse primer 5’ AACTCAAGGCTTGGTAGATC 5’; the bold C in the forward primer indicates a mutation, creating an NCO-I site). The polymerase chain reaction (PCR) conditions were as follows: five minute initial denaturation at 94°C, followed by 37 cycles (94°C for 30 seconds, 50°C for 30 seconds and 72°C for 30 seconds). The PCR products were digested with the restriction enzyme NCO-1 (New England BioLabs, MA) and separated on a 2.5% agarose gel stained with ethidium bromide.

For the determination of the TLR2 polymorphism, the DNA was amplified with primers (forward primer: 5’ GATGCATTGTGTCTTACAGTG 3’ and reverse primer: 5’ TGCACCCTCACTCTTCA A 5’). The PCR was as follows: five minute initial denaturation at 94°C, followed by 37 cycles (94°C for 30 seconds, 56°C for 30 seconds and 72°C for 30 seconds). The PCR products are digested with Aci-I (New England BioLabs, MA) and separated on a 2.5% agarose gel, stained with ethidium bromide.

Statistical analysis

Statistical evaluation was performed by using the Mann-Whitney test. Values were considered significant at p<0.05.

RESULTS

Ex vivo cytokine production

IFNγ and IL-10 production

In earlier experiments, we studied the kinetics of cytokine production, after stimulation with Candida or LPS, and we found that the proinflammatory cytokine production is maximal at 24 hours and that of IFNγ and IL-10 at 48 hours (data not shown). After 48 hours of stimulation with 10⁷ cfu/ml heat-killed C. albicans, the IFNγ production in patients was significantly lower than that in controls (figure 1A; p<0.02). In contrast, the IL-10 production in patients with CMC tended to be greater than that in controls (figure 1A; p>0.05). When the cells were stimulated with a lower amount of C. albicans (10⁶ cfu/ml), the results were similar, showing a lower production of IFNγ in patients compared with controls (data not shown). When diluted whole blood was incubated with either E. coli LPS (1 ng/ml) or PHA (10 μg/ml) for 48 hours, there was a tendency toward lower production of IFNγ upon stimulation with either LPS or PHA in the patient group in comparison with the control group (figure 2, p>0.05). A similar trend was observed after 24 hours of incubation with either stimulus (data not shown).
Figure 1
Diluted whole blood of patients with CMC (n=7) and healthy controls (n=14) was stimulated with heat-killed C. albicans (10⁷ cfu/ml) and production of IFNγ, IL-10 (A) and IL-1β, TNF, IL-1Ra (B) was assessed after 48 hours.

* Significant difference between patients and controls, p<0.02.

Figure 2
Diluted whole blood of patients with CMC (n=7) and healthy controls (n=14) was stimulated with LPS (A and B) or PHA (C and D) and production of IFNγ, IL-10 and IL-1β, TNF, IL-1Ra was assessed after 48 hours.

No significant differences between patients and controls were found.
TNF, IL-1β and IL-1Ra production
Diluted whole blood of patients with CMC and healthy volunteers was incubated with heat-killed *C. albicans*, and the cytokine response was analysed after 24 and 48 hours (figure 1B). After 24 hours of incubation, there were no significant differences in the production of IL-1β, IL-1Ra and TNF between patients and controls (table 1). Similarly, there was no significant difference in production of TNF, IL-1β or IL-1Ra between patients with CMC and healthy controls when stimulated with either LPS or PHA for 24 hours (table 1) or 48 hours (figure 2). In all experiments, the cytokine production in the absence of specific stimuli was very low, and no significant differences were observed between patients and controls.

Toll-like receptor 2 and 4 polymorphisms
The TLR4 Asp299Gly polymorphism and the TLR2 Arg677Trp polymorphism were assessed in blood. In the healthy control population (200 subjects), TLR4 polymorphism was present in 21 cases (11%), whereas none of them had a TLR2 polymorphism. Of the seven CMC patients, two (father and son) were heterozygous for the TLR4 Asp299Gly mutation. None of the CMC patients were positive for the TLR2 Arg677Trp mutation. After *C. albicans* stimulation, lowest IFNγ production was seen in the two patients with the TLR4 mutation. One of these patients had a IFNγ concentration below the detection limit, in the other heterozygous patient, IFNγ was 28 pg/ml, versus 340 ± 155 pg/ml in the other CMC patients and 2279 ± 609 pg/ml in healthy controls.

**DISCUSSION**

In our study of patients with CMC, we investigated cytokine production in whole blood stimulated with specific microbial stimuli such as *C. albicans*, or LPS, and PHA, a direct stimulus of T cells. After stimulation with *C. albicans*, IFNγ production was 70 to 90% lower in CMC patients as compared with healthy controls. In contrast, the production of the anti-inflammatory cytokine IL-10 tended to be higher in CMC patients, whereas no difference in the release of TNF, IL-1 and IL-1Ra was seen between CMC patients and healthy volunteers.

The defective IFNγ release appeared to be rather specific for candidal stimulation. Microbial components stimulate IFNγ production through intermediary release of monocyte products such as IL-12 and IL-18, while PHA directly stimulates T lymphocytes. Thus, the difference between *Candida* and PHA stimulation suggests that the defect in CMC patients may be localised at the level of monocyte. In contrast to the release of IFNγ, production of IL-10 upon stimulation of whole blood with *Candida* tended to be higher in CMC patients compared with controls. As IL-10 is a potent anti-inflammatory cytokine which counteracts the actions of IFNγ, the IFNγ/IL-10 ratio is considered to be important in defence against *C. albicans*. Therefore, the greater release of IL-10 in CMC patients further contributes to a reduced IFNγ/IL-10 ratio and is likely to also be involved in the defective activation of anticandidal mechanisms.

Our data are in accordance with those of Gravenor et al. demonstrating higher IL-10 levels and deficient IL-12 production in CMC patients after *C. albicans* stimulation, whereas the expression of the IL-12 receptor appears to be normal in CMC patients. Since there was a tendency for lower IFNγ production after stimulation with PHA, an additional defect at the level of the T lymphocyte cannot be ruled out. Not all studies in CMC patients have observed decreased Candida-specific IFNγ release. The difference between these studies and ours probably lies in the experimental conditions: we used a whole-blood stimulation, whereas the other studies used cultures of isolated PBMC, in which IFNγ production may be sub-optimal.

Additional studies have also reported increased release of other anti-inflammatory cytokines such as IL-4 and IL-6. All these data suggest a strong Th2 bias in patients with CMC. Several experimental studies have demonstrated the deleterious effects of Th2-like cytokines for the antifungal defence, in contrast to the beneficial effects of Th1 cytokines.

Two out of seven CMC patients were heterozygous for the TLR4 Asp299Gly polymorphism, whereas the TLR2 Arg677Trp polymorphism was detected in none of the CMC patients. In the general population, the incidence of

<table>
<thead>
<tr>
<th>CYTOKINE</th>
<th>RPMI CONTROLS</th>
<th>C. ALBICANS CONTROLS</th>
<th>LPS CONTROLS</th>
<th>PHA CONTROLS</th>
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<tbody>
<tr>
<td>TNF (ng/ml)</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>IL-1β (ng/ml)</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>1.1 ± 0.2</td>
<td>0.1 ± 0.04</td>
</tr>
<tr>
<td>IL-1Ra (ng/ml)</td>
<td>0.8 ± 0.2</td>
<td>1.2 ± 0.6</td>
<td>3.2 ± 0.3</td>
<td>2.6 ± 0.4</td>
</tr>
</tbody>
</table>

Whole blood of CMC patients (n=7) and healthy controls (n=14) was stimulated with different stimuli. Values are given as means ± SEM.

Van der Graaf, et al. IFNγ and TLR in candidiasis.
the TLR4 mutation varies between 6 and 11%,\(^8\) whereas the TLR2 mutation is very rare, although only limited data are available.\(^9\) The present study is limited due to the small number of patients. Therefore, no epidemiological conclusions can be drawn from the observation on TLR polymorphisms. The only conclusion to be made is that the TLR polymorphisms that have been identified so far are not the major cause of the immunological abnormalities in CMC patients, since not all of the CMC patients had the polymorphism. Interestingly, the two patients with the TLR4 polymorphism had the lowest IFN\(_\gamma\)/H\(_9253\) production on Candida stimulation among all tested individuals. This suggests that TLR4 plays a role in Candida-specific IFN\(_\gamma\)/H\(_9253\) production.

In conclusion, our results show an imbalance in the cytokine network in CMC patients using Candida stimulation. The defective IFN\(_\gamma\) production is likely to be involved in the chronic infections with Candida species. The molecular defect responsible for this syndrome still needs to be localised. However, the known Toll-like receptor 2 and 4 polymorphisms do not play a crucial role in pathogenesis of this disease.

REFERENCES


Advertentie Thyrax

Van der Graaf, et al. IFN\(_\gamma\) and TLR in candidiasis.
A patient with pancytopenia and microcytic megaloblastic anaemia

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CASE REPORT

A 40-year-old woman was referred to our outpatient clinic with suspected myelodysplastic syndrome. Her symptoms had started six months ago and consisted of fatigue, a sore tongue, painful toes and fingers, and weight loss of more than 10 kg despite normal appetite and a non-vegetarian diet. Furthermore, she bruised easily and her family observed that she looked pale. The menses and stools were normal. Her additional medical history only revealed several periods of iron suppletion as a child because of anaemia without further analysis. She was not taking any medication and did not use alcohol. The family history revealed a grandfather and an aunt on her father’s side with vitamin B12 deficiency. Physical examination showed a pale woman with dyed hair, a glossitis with a solitary lesion on the tongue and a slightly enlarged spleen. Blood analysis revealed a pancytopenia with a microcytic hypochromic anaemia (figure 1) (with a haemoglobin level of 3.9 mmol/l, MCV 75 fl and reticulocytes 1%), total leucocytes of 2.0 x 10⁹/l with a normal differential count and platelets of 25 x 10⁹/l, as well as low levels of vitamin B12 (0.05 nmol/l, normal range 0.15 to 0.70) and folate (6 nmol/l, normal lower limit 12) but a normal iron status. The haemolytic parameters were increased, with a total bilirubin of 49 µmol/l, LDH 11,300 U/l, haptoglobin <0.02 g/l and a negative antiglobulin test.

A bone marrow aspirate showed a hypercellular bone marrow with megaloblastic cells, moderate haemophagocytosis and normal iron load (figure 2). The peripheral blood smear also showed megaloblastic features, including neutrophilic hypersegmentation (figure 1). Analysis of possible malabsorption and autoimmune disorders showed no abnormalities; however, parietal cell antibodies were positive. Biopsies taken from the stomach showed features of chronic inflammation. The Schilling test was also obviously disturbed after adding intrinsic factor.

Therefore, we have a woman with microcytic anaemia, but obvious megaloblastic features in the peripheral blood smear and bone marrow aspirate.

WHAT IS YOUR DIAGNOSIS?

What is this woman suffering from and what is your clinical interpretation?
See page 389 for the answer to this photo quiz.

A colour version of this photo quiz can be found on our website www.njmonline.nl.
Unexpected prolonged extreme hypocalcaemia and an inadequate PTH response in a patient with metastatic breast carcinoma

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ABSTRACT

Although hypercalcaemia is often encountered during the course of malignant disease, hypocalcaemia appears to be rather rare. We describe a 37-year-old patient with metastatic carcinoma of the breast, who developed extreme hypocalcaemia (as low as 0.75 mmol calcium per litre) after chemotherapy. This is caused by a combination of hungry-bone syndrome and an insufficient parathyroid response. The latter may be the result of a direct toxic effect of chemotherapy on parathyroid hormone (PTH) synthesis possibly in combination with microscopic tumour infiltration in the parathyroid glands. Correction of the extreme hypocalcaemia over a period of 100 days by oral and intravenous calcium supplementation, corresponding to a total of 352 gram elemental calcium (1/3 of the total body calcium), resulted in gradual symptomatic relief. The possible mechanisms for these findings are discussed and the literature is briefly reviewed.

INTRODUCTION

Hypercalcaemia due to carcinoma metastatic to bone occurs frequently. In contrast, hypocalcaemia is a rare complication of breast and prostate carcinoma.1-6 A number of factors may be implicated in the development of hypocalcaemia in cancer patients, including hypalbuminaemia, surgical and infiltrative hypoparathyroidism, radiologically destructed parathyroid glands, osteoblastic metastases, hypomagnesaemia, vitamin D deficiency, renal failure, massive cell lysis, drug effect and sepsis. We describe a patient with advanced breast carcinoma who developed extreme hypocalcaemia due to the combination of osteoblastic metastases and an inadequate PTH response.

METHODS

The Vitros 950 analytical system (Ortho Clinical Diagnostics) was used for the determination of creatinine, calcium, magnesium, albumin, inorganic phosphorus, alkaline phosphatase (AP), blood urea nitrogen, γ-glutamyl transferase (γ-GT), lactate dehydrogenase (LDH), aspartate aminotransferase, alanine aminotransferase in plasma, and for calcium in urine. Serum ionised calcium was measured with an ion-selective electrode on the Synthesis analyser (Instrumentation Laboratory). Thyroid-stimulating hormone, PTH and tumour markers were determined in serum on the Immuno I analyser (Bayer Diagnostics), the Immulite system (DPC) and IMx (Abbott), respectively. Cation exchange chromatography was used for the determination of hydroxyproline in urine. PTH-related peptide (PTHrP), calcitonin, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D were determined by competitive radioimmunoassays. All instruments and assays were calibrated and operated according to manufacturer’s recommendations.

CASE REPORT

A 37-year-old woman was admitted because of progressive mental instability and paresthesia of the distal extremities.
Her medical history included breast carcinoma (T2N1M0) for three years, for which she had undergone a mastectomy, radiotherapy and chemotherapy (cyclophosphamide, methotrexate and 5-fluorouracil). Six months before admission, sacroiliac metastases were discovered and radiotherapy, goserelin and tamoxifen were given. Two months before admission, thoracic spine metastases were irradiated and pamidronate (90 mg iv) was administrated. Four days before admission, FEC chemotherapy (5-fluorouracil, epi-adriamycin and cyclophosphamide) was started because of progressive painful osteoblastic metastases and rising tumour markers (CA15-3 46 kU/l and CEA 25.3 μg/l). On admission, physical examination showed multiple skin metastases, a heart rate of 90 beats/min and blood pressure of 100/70 mmHg. Plasma calcium and phosphate levels were normal (figure 1 and table 1).

Four days later, she became profoundly hypocalcaemic (1.26 mmol/l) and oral calcium supplementation was started. An MRI scan of the parathyroid region did not show any abnormalities. Serum calcium levels dropped to 1.09 mmol/l on day 12 and intravenous calcium supplementation was added. Nevertheless, the calcium level decreased to 0.75 mmol/l at day 21. Around this time, she suffered from distal paresthesia, severe cramps in her face, arms, legs and abdomen, as well as chest tightness. Other biochemical results were phosphate 2.61 mmol/l, ionised calcium 0.55 mmol/l, AP 1005 E/l and LD 1309 μl. Serum parathyroid hormone level was 1.1 pmol/l. Plasma albumin and magnesium, and serum PTHrP and calcitonin concentrations, as well as renal, liver and thyroid functions were normal. The concentration of 25-hydroxyvitamin D was normal. However, its active metabolite 1,25-dihydroxyvitamin D was elevated, indicating an adequate metabolism of vitamin D due to hypocalcaemia. Urinary calcium excretion was 0.72 mmol/day, hydroxyproline 0.20 mmol/day/m².

A second course of FEC chemotherapy was given at day 22. Despite the normal levels of both vitamin D and magnesium, calcitriol (5 μg oral) and magnesium sulphate (2 grams iv) were added from this moment. However, this did not result in a subsequent rise in calcium concentration. The PTH level fell even further (table 1). At day 50 a third course of chemotherapy was given in which epi-adriamycin was replaced by methotrexate.

After 100 days of excessive calcium supplementation, her symptoms gradually improved. The cumulative amounts of calcium administrated were: oral calcium carbonate 242 gram and intravenous calcium glubionate 1680 gram, corresponding to 352 gram elemental calcium (1/3 of the total body calcium). She was discharged from the hospital with a calcium of 1.43 mmol/l, decreasing tumour marker.

![Figure 1](image_url)

**Figure 1**

*Time course of plasma calcium and alkaline phosphatase concentrations*

Total calcium supplementation: 242 gram calcium carbonate orally and 1680 gram calcium glubionate intravenously corresponding to a total of 352 gram elemental calcium. With normal renal and liver functions alkaline phosphatase represents a marker of osteoblastic activity. Bars indicate normal ranges for calcium (2.10-2.70 mmol/l) and alkaline phosphatase (<120 μl). Arrows indicate chemotherapy and calcitriol administered. Chemo means the start of a course of chemotherapy.
Table 1
Laboratory parameters at day 0, 4, 21, 33, 72 and 101 after admission

<table>
<thead>
<tr>
<th></th>
<th>DAY 0</th>
<th>DAY 4</th>
<th>DAY 21</th>
<th>DAY 33</th>
<th>DAY 72</th>
<th>DAY 101</th>
<th>REFERENCE VALUES</th>
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<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Blood urea nitrogen</td>
<td>2.8</td>
<td>3.6</td>
<td>4.1</td>
<td>1.7</td>
<td>2.2</td>
<td>2.5-7.0 mmol/l</td>
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<tr>
<td>Creatinine</td>
<td>32</td>
<td>30</td>
<td>39</td>
<td>40</td>
<td>38</td>
<td>50-90 μmol/l</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>275</td>
<td>123</td>
<td>1005</td>
<td>671</td>
<td>159</td>
<td>133</td>
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<tr>
<td>γ-GT</td>
<td>80</td>
<td>64</td>
<td>43</td>
<td>32</td>
<td>&lt;35 U/l</td>
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<tr>
<td>LD</td>
<td>1534</td>
<td>879</td>
<td>1309</td>
<td>714</td>
<td>504</td>
<td>&lt;500 U/l</td>
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<td>Calcium</td>
<td>2.58</td>
<td>1.26</td>
<td>0.75</td>
<td>1.18</td>
<td>1.50</td>
<td>1.32</td>
<td></td>
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<td>Ionised calcium</td>
<td>0.40</td>
<td>0.64</td>
<td>0.74</td>
<td>0.85</td>
<td>1.15-1.35 mmol/l</td>
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<td>Organic phosphate</td>
<td>1.36</td>
<td>1.49</td>
<td>2.61</td>
<td>1.67</td>
<td>1.98</td>
<td>2.23</td>
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<td>Albumin</td>
<td>32</td>
<td>33</td>
<td>35</td>
<td>38</td>
<td>35</td>
<td>35-50 g/l</td>
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<tr>
<td>Magnesium</td>
<td>0.67</td>
<td>0.72</td>
<td>0.87</td>
<td>0.74</td>
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<td>25(OH) Vitamin D</td>
<td>31</td>
<td>70</td>
<td>&lt;140</td>
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<td>120</td>
<td>20-100 pmol/l</td>
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<tr>
<td>1,25(OH) Vitamin D</td>
<td>193</td>
<td>126</td>
<td>126</td>
<td>126</td>
<td>126</td>
<td>48-161 pmol/l</td>
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<td>PTH</td>
<td>1.1</td>
<td>0.3</td>
<td>0.2</td>
<td>0.9</td>
<td>1.0-6.0 pmol/l</td>
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<tr>
<td>Calcitonin</td>
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<td>&lt;0.14 μg/l</td>
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<td>TSH</td>
<td>2.19</td>
<td>3.4-6.0 μU/l</td>
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<td>CEA</td>
<td>25.3</td>
<td>18</td>
<td>&lt;5 μg/l</td>
<td></td>
<td></td>
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<tr>
<td>CA15-3</td>
<td>46</td>
<td>31</td>
<td>&lt;30 kU/l</td>
<td></td>
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<tr>
<td>PTHrP</td>
<td>0.4</td>
<td>&lt;2.6 pmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>24-hour urine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Calcium</td>
<td>0.72</td>
<td>0.96</td>
<td>1.60</td>
<td>&lt;0.61</td>
<td>2.5-10.0 mmol/24h</td>
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<tr>
<td>Hydroxyproline</td>
<td>0.20</td>
<td>0.28</td>
<td>0.36</td>
<td>0.28</td>
<td>0.05-0.17 mmol/24h/m²</td>
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</table>

γ-GT = γ-glutamyl transferase, LDH = lactic dehydrogenase, PTH = parathyroid hormone, TSH = thyroid-stimulating hormone, CEA = carcinoembryonic antigen, CA 15-3 = a tumour marker for breast carcinoma, PTHrP = PTH-related peptide.

levels, disappearing osteoblastic metastatic activity on radiological investigation and reduction in number and severity of skin metastases. In the next months, her serum calcium and PTH concentrations returned to normal (2.11 mmol/l and 3.6 pmol/l, respectively) and calcium supplementation was discontinued.

Five months later, she presented with symptoms of hypercalcaemia. Her laboratory results showed a serum calcium of 3.00 mmol/l and an undetectable PTH level (<0.1 pmol/l). This time, X-ray investigation showed numerous new osteolytic metastases. Two months later, she died. Permission for autopsy was denied.

**DISCUSSION**

Hypercalcaemia is a common complication in patients with carcinoma metastatic to bone. Hypocalcaemia is an uncommon but not unexpected finding in association with osteoblastic bone metastases, most commonly associated with metastases of prostate and breast carcinomas. In 1984, Pepper et al. reported the first full endocrinology evaluation of a patient with osteoblastic metastases from a primary lesion in the breast. This evaluation demonstrated that patients with osteoblastic metastases have an increased calcium resorption by the bone. The extreme hypocalcaemia in our patient is to our knowledge the lowest calcium concentration ever reported in this category of patients (Table 2). From the possible causes of hypocalcaemia, hypoalbuminaemia, renal insufficiency, hypomagnesaemia, pancreatitis and vitamin D deficiency could be excluded. Hypocalcaemia may be the result of the ‘hungry-bone syndrome’ after parathyroidectomy and/or hyperparathyroidism. In the first case, the PTH is low and in the latter case, PTH is increased. In case of osteoblastic bone metastases or acute mineralisation after tumour-lysis syndrome, a secondary hyperparathyroidism will develop. In our patient the PTH was low and remained low for a long time. Therefore, there must have been a primary hypoparathyroidism from the beginning, which could be a hypoparathyroidism caused by an autoimmune process, after parathyroidectomy or tissue destruction by infiltrating tumour cells or by irradiation. The last mentioned is a possible cause, because two months before admission the thoracic spine of the patient was irradiated. However, the radiation-exposure fields suggested that radiation injury...
of the parathyroid glands was unlikely. Neck surgery was not performed in our patient. The incidence of metastatic involvement of the parathyroid glands in cancer patients confirmed by autopsy is 6 to 12%. However, parathyroid metastases will only lead to hypoparathyroidism when at least 70% of the parathyroid glandular tissue is replaced by metastatic tumour cells. So, diffuse metastatic infiltration of the parathyroid glands could have led to the diminished PTH synthesis in our patient. Although she had several skin metastases, an MRI scan of the neck did not show any abnormalities of the parathyroid glands. Microscopic infiltration of the parathyroids, however, cannot be excluded because permission for autopsy was denied. However, if present, these micrometastases could have successfully responded to the chemotherapy as the skin metastases had done. Among the 13 published patients with breast carcinoma and documented hypocalcaemia and hypoparathyroidism (table 2), parathyroid metastases could be identified in only five patients. Autopsy did not disclose diffuse metastatic infiltration of the parathyroid glands in only one case. In the other seven cases parathyroid examination was not performed. Grieve et al. described three patients with osteolytic metastases of breast cancer who initially had symptomatic hypercalcaemia, but after chemotherapy developed hypocalcaemia and an inappropriate PTH response. In all three patients, the inadequate PTH response was transient as evidenced by a gradual normalisation of the PTH levels. Tumour lysis can lead to the release of excessive amounts of phosphate with hypocalcaemia as a result. Phosphate levels were found in the first stage of the hypocalcaemic episode. However, a contributory role of tumour lysis to the hypocalcaemia cannot fully be excluded, because hyperphosphataemia was found later on. Urinary calcium excretion was low, so we can only postulate that the patient’s osteoblastic metastases rapidly absorbed calcium. Ectopic secretion of calcitonin or PTHrP by tumour cells was unlikely in our patient because of normal calcitonin and PTHrP concentrations and normal calcium levels on admission.

The utility of biphosphonates is well established, not only in the treatment of tumour-associated hypercalcaemia, but also to relieve pain in normocalcaemic patients with bone metastases. Severe hypocalcaemia has been described as a complication of pamidronate therapy in a hypercalcaemic patient and in a normocalcaemic patient with bone metastasis due to breast carcinoma. Although the PTH failed to rise after biphosphonate administration in these patients with subclinical hypoparathyroidism resulting in prolongation of the hypocalcaemia, no mechanism is known by which biphosphonates can cause a latent hypoparathyroidism, as was suggested to be the cause of hypocalcaemia in these case reports. Pamidronate is unlikely to be the cause of the hypocalcaemia in our patient because the effect of pamidronate is to be expected within two days after administration, and because the calcium level on admission was normal. Epi-adriamycin and other chemotherapeutic agents might cause hypocalcaemia directly by suppressing PTH

<table>
<thead>
<tr>
<th>REFERENCE</th>
<th>SERUM CALCIUM (MMOL/L)</th>
<th>SERUM PHOSPHATE (MMOL/L)</th>
<th>BONE METASTASES</th>
<th>PARATHYROID EXAMINATION</th>
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<tr>
<td>Bouvier et al.1</td>
<td>2.20</td>
<td>1.20</td>
<td>Osteoblastic</td>
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<tr>
<td>Unger et al.2</td>
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<tr>
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<td>2.25</td>
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</tr>
<tr>
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<td>1.57</td>
<td>1.42</td>
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<tr>
<td>Mariette et al.5</td>
<td>1.36</td>
<td>3.06</td>
<td>Medullary metastases</td>
<td>Parathyroid metastases</td>
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<td>Wantanabe et al.6</td>
<td>1.35</td>
<td>1.58</td>
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<td>Parathyroid metastases</td>
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<tr>
<td>Comlekci et al.7</td>
<td>1.32</td>
<td>1.96</td>
<td>Not reported</td>
<td>Not done</td>
</tr>
<tr>
<td>Wiegand et al.8</td>
<td>1.30</td>
<td>1.23</td>
<td>Osteoblastic</td>
<td>Not done</td>
</tr>
<tr>
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<td>1.28</td>
<td>1.46</td>
<td>Mixed</td>
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</tr>
<tr>
<td>Hall et al., case 9</td>
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<td>2.20</td>
<td>Osteoblastic</td>
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</tr>
<tr>
<td>Grieve et al., case 3</td>
<td>1.20</td>
<td>1.60</td>
<td>Mixed</td>
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<tr>
<td>Grieve et al., case 1</td>
<td>1.08</td>
<td>1.50</td>
<td>Mixed</td>
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</tr>
<tr>
<td>Present case</td>
<td>0.75</td>
<td>2.61</td>
<td>Osteoblastic</td>
<td>Not done</td>
</tr>
</tbody>
</table>

*The lowest calcium concentration of a case reported in the reference is noted.*
secretion.20,21 This could be the cause in our case of hypocalcaemia because the parathyroid glands proved to be able to excrete PTH up to normal levels three months after her last chemotherapy. Moreover, calcium concentration started to increase from day 60, ten days after her last chemotherapy in which epi-adriamycin was replaced by methotrexate. Furthermore, adequate calcium and calcitriol supplementations are capable of maintaining calcium levels in the presence of an inappropriate PTH concentration and will not result in a further decline in calcium level as chemotherapy is continued. Alternatively, aminoglycosides such as adriamycin can induce renal tubular dysfunction leading to the loss of cations, such as calcium and magnesium. However, no hypomagnesaemia was found and calcium excretion was low.

In summary, we describe a patient with extensive osteoblastic metastases of breast cancer. We speculate that the combination of cytotoxic drugs, possible micrometastases present in the parathyroids and the ‘hungry-bone syndrome’ caused the extreme, prolonged hypocalcaemia and the inadequate PTH response in our patient. At the end of her admission, tumour load was decreased by chemotherapy, indicated by decreasing tumour markers, reducing skin metastases and disappearing osteoblastic metastatic activity on X-ray re-investigation. This resulted in decreased calcium utilisation by the metastatic process and improved clinical condition.

ACKNOWLEDGEMENT

We would like to thank Professor M.A. Blankenstein and P. Lips, from the Free University of Amsterdam, for their helpful suggestions while preparing the manuscript.

REFERENCES

ABSTRACT

Introduction: Epstein-Barr virus (EBV) establishes lifelong latent infection. In some patients the host-virus balance is disturbed, resulting in a chronic active EBV infection. The following case illustrates the difficulty in diagnosing and treating chronic EBV infection.

Case: A 30-year-old woman was referred because of recurrent swellings of lymphatic tissue of both eyelids, orbit and lymph nodes and general malaise since the age of 19. In the past, repeated biopsies showed MALT lymphoma and nonspecific lymphoid infiltrations. Now, a biopsy of an axillary lymph node showed paracortical hyperplasia with a polymorphous polyclonal lymphoid proliferation, and large numbers of EBV-encoded small RNA (EBER) positive cells, consistent with EBV infection. Laboratory investigation showed a high EBV viral load. No evidence of immunodeficiency was found. Chronic active EBV infection (CAEBV) was diagnosed. Treatment with high-dose acyclovir did not significantly reduce the viral load. Rituximab was given in an attempt to reduce the amount of EBV-infected B lymphocytes. However, soon after the second dose the patient died of a subarachnoidal haemorrhage.

Conclusion: This case report illustrates CAEBV as a rare manifestation of EBV-induced disease, which will be detected more frequently with the use of EBV-EBER hybridisation of lymph nodes and polymerase chain reaction (PCR) for EBV DNA. The prognosis is poor with no established therapeutic strategies.

INTRODUCTION

Almost every adult (90 to 95%) will have acquired Epstein-Barr virus (EBV) and will be seropositive for this herpes virus. The majority of primary infections pass unrecognised, but roughly 10% of EBV infections present as acute infectious mononucleosis, particularly in adolescence and adulthood.1 The oropharynx is thought to be the primary site of entry, where the virus binds to epithelial cells which are generally believed to be permissive for viral replication.2,3 The latter has recently been disputed and it might well be that B lymphocytes in the oropharynx instead of epithelial cells are the primary reservoir for replication as well as viral latency.4-6 EBV survives by maintaining a delicate balance with the host resulting in a latent infection,7 restricted to B lymphocytes. Sometimes, also T lymphocytes, epithelial cells and myocytes can be infected, usually with expression of a restricted set of latent gene products.8 Spread to new hosts is ensured by intermittent reactivation and productive replication at epithelial surfaces.9

Several patterns of latency have been recognised in which up to ten viral genes are expressed and are thought to be involved in establishing and maintaining the immortalised state of the infected cell. Six nuclear proteins belong to this group, of which EBNA-1 (Epstein-Barr virus nuclear antigen-1) is essential for episome replication and maintenance of the viral genome10 and EBNA-2 (Epstein-Barr virus nuclear antigen-2) for the process of B-lymphocyte immortalisation.11-13 Three membrane proteins belong to the latency state,
latent membrane protein-1 (LMP-1), LMP-2A (latent membrane protein-2A) and LMP-2B (latent membrane protein-2B). LMP-1 protects EBV-infected B cells from programmed cell death (apoptosis). LMP-2A and LMP-2B are integral membrane proteins which co-localise with LMP-1 in the plasma membrane of EBV-infected lymphocytes, EBV-encoded small RNAs (EBERs) are most abundantly present in latently infected B cells. Productive EBV replication results in expression of early antigens (EA), which are part of the replication machinery, and viral capsid antigens (VCA), which are structural constituents of the virion itself. The first antibodies produced during primary EBV infection, such as infectious mononucleosis, are IgM and IgG antibodies against VCA and EA, which can be detected together with the appearance of circulating atypical lymphocytes and heterophile antibodies. Increase of pre-existing IgG antibody titres against VCA and EA indicate reactivation of EBV infection. Antibodies against EBNA are usually produced somewhat later in time, e.g. during convalescence, but many exceptions exist where EBNA antibodies are found together with those against EA and VCA. Figure 1 shows the expression of different viral antigens and antibodies during primary EBV infection, during latency and during chronic active EBV infection (figure 1). Because EBV is a persisting virus, it must have strategies to elude the immune system. EBV-specific cytotoxic T lymphocytes (CTL) are thought to constitute the most important defence against EBV infection. However, the latency-associated protein EBNA-1 has evolved into a protein that escapes antigen processing (proteasome degradation, an essential step to form peptides, which can be presented in the context of HLA molecules to the immune system) and thus recognition by CTL, thereby promoting EBV latency, while immune surveillance by CTL can still abort viral proliferation. The EBV BCRF1 protein shares 70% of its amino acid sequence with interleukin-10 and can mimic the activity of IL-10 by inhibiting the interferon-γ synthesis by human peripheral blood mononuclear cells in vitro. The EBV BARF1 protein can inhibit the expression of interferon-α by monocytes. Interferon-γ and interferon-α inhibit the
outgrow of EBV-infected cells, so BCRF1 and BARF1 proteins probably help the virus to evade the host’s immune system during acute EBV infection or reactivation of virus from latency.  

EBV is associated with a large range of inflammatory and proliferative diseases as summarised in table 1. Chronic active EBV infection (CAEBV infection) is one of the manifestations of EBV-induced disease. The following case illustrates the difficulty in diagnosing and treating CAEBV.

**Case Report**

A 30-year-old female was referred to the internal medicine department with recurrent unexplained orbital swelling and general symptoms. At the age of 19 she developed the first swelling in the left orbit and eyelid. A biopsy was nonconclusive because of extensive damage to the tissue. No additional therapy was instituted. At the age of 22 a similar swelling developed in the left orbit and eyelid. A biopsy of the mass in the eyelid showed a monotonous lymphoplasmacellular infiltration without light chain restriction (>10 times more lambda than kappa positive cells) consistent with a diagnosis of low-grade malignant orbital MALT lymphoma. Another biopsy in the same year, taken from the left orbita, showed lymphoid follicles with germinal centres and a lymphoplasmacellular proliferation with only moderate prevalence of lambda positive cells, consistent with a reactive lymphoid hyperplasia. EBV staining was not performed and no material for additional staining was available. She was treated with prednisone and when the swelling increased 18 months later, at the age of 24 years, she received local radiotherapy. At the age of 27, she was seen in our hospital to find an explanation for the increasing exophthalmus of her left eye. No indication was found for thyroid disease. A cerebral CT scan showed no abnormalities, and in particular no protrusio bulbi. A wait-and-see policy was adopted.

On referral she presented with a palpable mass in her right eyelid which had become obvious during the previous week. She was 14 weeks pregnant. She complained of fatigue and night sweats, but had no fever. Physical examination showed a palpable mass in the right eyelid of 0.8 x 0.2 cm, a protruding eye-bulb and firm, elastic submandibular and cervical lymph nodes up to 2 cm in diameter. No enlarged lymph nodes were found at other stations. Physical examination of heart, lungs and abdomen revealed no abnormalities. At that time, hybridisation for EBERs was not performed. Since a MALT lymphoma of the left orbita and eyelid has been diagnosed eight years before, lymphoma staging was done. Sternal aspirate biopsy of the left orbita, and crista biopsy showed no localisation of a malignant lymphoma. Ultrasound of the abdomen and chest X-ray showed no intra-abdominal or mediastinal lymphomas. During the last month of her pregnancy, the cervical and axillary lymph nodes increased in size. Several weeks after an uncomplicated delivery and the birth of a healthy child she complained of progressive fatigue, arthralgia without signs of active arthritis and volatile erythematous skin lesions. Pathological examination of a skin biopsy revealed a focal increase in lymphocytes perivascularly, not meeting criteria for the diagnosis of vasculitis. CT scanning...
of chest and abdomen showed axillary, mediastinal, retroperitoneal and iliacal lymph node proliferation, but all smaller than 1 cm. Biopsy of an axillary lymph node showed, apart from reactive follicles, a predominantly paracortical hyperplasia with large atypical cells among which many large B cells and positive hybridisation for EBERs. LMP-1 staining was negative. This finding is consistent with a histological diagnosis of EBV-induced lymphoproliferation or infectious mononucleosis. Three months later the exophthalmus of her right eye rapidly progressed and there was further enlargement of the lymph nodes. Prednisone (1 mg/kg) treatment was initiated. The exophthalmus and lymph nodes completely disappeared, but recurred when prednisone was tapered off. CT scanning of the right orbita revealed a soft tissue mass around the lacrimal gland. A second biopsy of an enlarged (cervical) lymph node was taken after the prednisone was stopped. Histology showed extensive paracortical and perisinusoidal infiltration of lymphocytes, plasma cells and eosinophils with scattered large activated lymphocytes and hybridisation for EBERs was positive, predominantly in the immunoblast-like cells. Now also LMP-1 staining was positive. The findings were grossly identical to the biopsy five months earlier. Because EBERs and LMP-1 were found in the lymph node biopsy more extensive EBV serology was performed. This showed an elevated titre of VCA-IgG of 512 E/ml, an EA-IgG of 128 E/ml, EBNA-IgG of 32 E/ml. The VCA-lgM was negative. Furthermore a high EBV viral load was measured by quantitative PCR: 10^4 genome equivalents (GEQ)/ml. Chronic active EBV infection (CAEBV) was cause of lymphadenopathy was considered, because EBV viral loads were high and there was a persisting lymphadenopathy with B-lymphocyte proliferation and expression of EBERs. We did several investigations to exclude an immunodeficiency. The total amount of T cells was low (0.58 x 10^9/l), but the CD4/CD8 ratio was normal (1.94). In vitro T-lymphocyte stimulation with phorbol myristate acetate (PMA) was normal, with normal production of IL-2, IL-4 and interferon-γ. There were normal numbers of B cells and natural killer (NK) cells. The CD4/CD45RA versus RO ratio was less than 1, suggesting less activated and naive T cells compared with memory T cells. Treatment with high-dose acyclovir (6 g/day orally) for three months resulted in a limited reduction of EBV viral load to 6 x 10^4 GEQ/ml whereas the clinical symptoms increased. Rituximab (anti-CD20-antibodies) treatment (375 mg per dose) was given in an attempt to reduce the amount of EBV-infected B lymphocytes and to improve the clinical condition. However, six days after the second dose the patient was found comatose. Cerebral CT scanning showed subarachnoidal haemorrhage. There was no evidence of lymphoma localisation or an infectious focus. She died the same day, post-mortem evaluation was not allowed.

**Discussion**

This case report describes a young woman who presented with recurrent periorbital swelling when she was 19 years of age. A low-grade malignant orbital MALT lymphoma was diagnosed on a biopsy from the orbital swelling when she was 23 years. Progressive symptoms during her first pregnancy and lymphadenopathy at the age of 30 were a reason for referral to our clinic for further evaluation. A lymph node biopsy showed a reactive histological picture consistent with viral infection and strong positive hybridisation for EBERs. This, together with the high plasma EBV titres, made us consider the diagnosis CAEBV, according to the criteria developed by Straus.26 Straus defined three main criteria for the diagnosis CAEBV:

- Severe illness of greater than six months duration which began as a primary EBV infection or was associated with grossly abnormal EBV-antibody titres (lgG to VCA >1:5120; antibody to EA >1:5640; or antibody to EBNA <1:2).
- Histological evidence of major organ involvement such as interstitial pneumonia, hypoplasia of some bone marrow elements, uveitis, lymphadenitis, persistent hepatitis or splenomegaly.
- Detection of increased quantities of EBV in affected tissues.

CAEBV is characterised by chronic or recurrent infectious mononucleosis-like symptoms persisting over a long period. The difference with latent EBV is viral replication and thus the presence of replicative antigens. In general, patients with this disease have no evidence of any prior immunological abnormality, as was the case in our patient. The pathogenesis of CAEBV is still unknown. There seems to be a deficit in the specific T-cell response against EBV, but not a general immune deficiency. The interaction between EBV and adenovirus probably promotes the development of CAEBV by reducing the expression of human histocompatibility class 1 complex by adenovirus and transient immune suppression during acute EBV infection.27-28 CAEBV is associated with the development of malignant lymphoma, especially T-cell lymphoma.29 CAEBV is a disease with a high morbidity and high mortality: The probability of five-year survival is 0.45 for older patients (≥8 years) and 0.94 for younger patients.29 Our patient met the main criteria of Straus. Although the EBV-antibody titres of this patient did not meet the first criterion defined by Straus, titres cannot be compared between laboratories, particularly because our laboratory uses a test which deliberately results in low titres.30 Although the antibody titres against EBV were elevated, the pattern of antibodies in this patient was normal, notably, with antibodies against EBNA being
present. This is in contrast with Miller’s report that patients with CAEBV have no detectable antibodies against EBNA. All the symptoms observed in our patient are consistent with CAEBV as is shown in table 2.

Table 2
Symptoms of chronic active Epstein-Barr virus infection (CAEBV)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-grade fever</td>
<td>Intestinal perforation</td>
</tr>
<tr>
<td>High fever (T-cell type)</td>
<td>Large vessel arteritis</td>
</tr>
<tr>
<td>Sepsis</td>
<td>Coronary artery aneurysm</td>
</tr>
<tr>
<td>Pancytopenia</td>
<td>Exophthalmus</td>
</tr>
<tr>
<td>Haemophagocytic syndrome</td>
<td>Uveitis</td>
</tr>
<tr>
<td>Malignant lymphoma</td>
<td>Cerebellar ataxia</td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>Panencephalitis</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>Calcification in basal ganglia</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>Polyneuropathy</td>
</tr>
<tr>
<td>Tubulo-interstitial nephritis</td>
<td>Hypersensitivity to mosquito bites (HMB) (NK-cell type)</td>
</tr>
<tr>
<td>Interstitial pneumonia</td>
<td>Hydroa vacciniforme-like eruptions</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>Erythema</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>Sicca syndrome</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>Oral ulcers</td>
</tr>
</tbody>
</table>

* Symptoms present in patient described in this case report.

It is important to note that nowadays Epstein-Barr virus concentrations can be measured in plasma by quantitative PCR. This new technique, of which the diagnostic significance is rapidly growing, showed values of up to $10^4$ GEQ/ml of the viral genome in blood, a value which strongly supports our diagnosis of CAEBV. At present atypical proliferations of lymphatic tissues are routinely stained on hybridisation for EBERs and LMP-1. Since quantitative PCR in plasma and tissue staining on EBV is possible it can be expected that the diagnosis of CAEBV will be made more frequently. Review of the criteria of CAEBV might be necessary, as Kimura et al. have also proposed. They propose that a viral load exceeding $10^{-5}$ GEQ/μg tissue DNA could be used as a diagnostic criterion for CAEBV. On the other hand, tissue EBV may be positive after EBV infection in normal individuals, while EBV plasma PCR levels should be negative. So we suggest the criterion of plasma, not tissue, PCR levels in the diagnosis of CAEBV.

Most but not all patients with CAEBV described in the literature have periods of low-grade fever. Our patient did not experience fever, but she complained of night sweats. Okano et al. describe 26 patients with severe active EBV infection (SCAEBV) of which three did not present with fever. Almost all patients described in the literature are relatively young. The mean age of onset in Kimura’s patient group was 8.3 years; the oldest patient was 27 years at onset of the disease. Our patient developed the first signs of the disease at the age of 19. The swelling of the left orbita in our patient, which was diagnosed as MALT lymphoma years before, might also be related to CAEBV, but unfortunately there is no material available for retrospective LMP1 and EBER staining. Low-grade malignant MALT lymphoma is in general a disease of the older age groups, but MALT lymphomas and low-grade plasmacytomas of the upper oropharynx, nasopharynx and orbit are not unusual in younger people in the third decade. Whether these low-grade malignant tumours are related to EBV infection is not known but needs further investigation.

Because CAEBV is a disease with a poor prognosis, several treatment strategies have been proposed. Administration of immune-modulating agents such as interferon-α or interleukin-2 have been described to restrain the clonal development of EBV-associated T-lymphoproliferative disease (T-LPD) and (B-LPD). It does not eradicate proliferation of EBV. Antiviral agents such as acyclovir, gancyclovir and vidarabine have been tried. Our patient was treated with a high dose of acyclovir resulting in some reduction in EBV viral load, but with no clearance and with no effect on clinical signs and symptoms. Treatment with etoposide-based regimens or adoptive transfer of EBV-specific cytotoxic T lymphocytes have shown promising results. Rituximab (anti-CD20 monoclonal antibody) has successfully been used in patients with EBV lymphoma after kidney and bone marrow transplantation, inducing clinical remissions. Because a B-cell proliferation was seen in biopsies of lymph nodes, treatment with rituximab was instigated to reduce the amount of EBV-infected B cells. Unfortunately, we were not able to evaluate this therapy due to her sudden death. We can only speculate whether her sudden death, due to subarachnoidal bleeding, was related to CAEBV or was merely a coincidence. CNS involvement such as panencephalitis and cerebellar ataxia in CAEBV have been described, but cerebral bleeding is not mentioned. Since coronary artery aneurysms and arteritis have been described in CAEBV it is possible that cerebral vascular complications were the cause of death in our patient.

In conclusion, CAEBV is a rare manifestation of EBV-induced disease. It is based on an ineffective T-cell response against the EBV-infected cells, not due to a more generalised immune deficiency. The prognosis is poor with no established therapeutic strategies. If a patient presents with variable unexplained symptoms which fit in the spectrum of symptoms of CAEBV, EBV
viral loads should be measured and tissue should be stained atypical proliferations of reactive lymphatic tissues are completed with measuring viral replication in (quantitative) PCR, it can be expected that the diagnosis of CAEBV will be made more frequently and review of the criteria of CAEBV might be necessary.

REFERENCES

11. Jones MD, Foster L, Sheedy T, Griffin BE. The EB virus genome in Daudi Burkitt’s lymphoma cells has a deletion similar to that observed in a non-transforming strain (P3HR-1 of the virus. EMBO J 1985;4(4):813-21.

ABOUT THE COVER

‘Studie van persoon’

Lex Loman

Lex Loman, the artist on this month’s cover, works and lives in Arnhem, the Netherlands. He also studied in Arnhem, at the Academy of Fine Arts. In addition to a series of individual expositions, he has shown his work at many group exhibitions in the region of Arnhem. Loman’s work is also presented in the offices of several companies, for example the PTT and AKZO Nobel, in Assen, Breda and Arnhem. Since 1 January 2000 he has been active as printmaker assistant of the ‘Plaats Maken’ Printmaking Workshop. An original print from this limited edition is available at a price of € 150. You can order the print at Galerie Unita, Rijksstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands or by e-mail: galerie-unita@planet.nl.

De Boer, et al. Chronic active Epstein-Barr virus infection.
Abstract

Medical textbooks are an important aid in the process of diagnosing and treating patients. Medical students use these books to acquire the skills necessary for this process, while medical teachers and experienced doctors use them for teaching these competences. We posed the question whether medical textbooks are structured in such a way that medical students are taught to structure knowledge and to make a differential diagnosis in a logical way. Five major textbooks were compared with regard to four clinical problems (gastrointestinal bleeding, anaemia, oedema and heart failure). The presentation appeared to be very variable in respect of logic and systematic arrangement. In fact, it was disappointing that even in well-reputed textbooks, a systematic approach is lacking. We feel there is a need for improvement, in order to facilitate the learning of medical students and to enhance their abilities in clinical problem solving.

Introduction

For clinicians, medical textbooks are an important source of information on diseases. Such books are used to check whether certain symptoms or signs fit into a clinical syndrome, to look for diagnostic or therapeutic strategies and sometimes for completion of a differential diagnosis. Most textbooks are extensive and difficult to use, but a good index and electronic versions with a search system have enhanced accessibility.

Medical students are stimulated to buy and use textbooks of the major medical disciplines and discouraged to use readers with copies of articles without an index. In modern medical curricula, histories of patients are already used in the first years of training, and thus medical textbooks are used more intensively. These clinical problems have to be analysed, and consequently, a motivated plan for diagnostic procedures and therapy has to be made. In addition, students are stimulated to look for additional information in the medical literature. Depending on the study progress, level of competence and the depth needed, a concise or comprehensive textbook is used. Apart from finding answers to specific questions, it is important that students learn to use textbooks. In this way similar clinical problems can be solved and in the long run students will be able to reproduce differential diagnoses by head.

A systematic arrangement of differential diagnoses may be based on anatomy, pathophysiology or epidemiology. For symptoms as pain or bleeding an anatomical approach is useful, whereas for signs as fever or shortness of breath a pathophysiological one is preferable. Sometimes a combination of approaches is necessary, especially in case of a more detailed differential diagnosis. Others plead for scheme-induced reasoning as an aid in the instruction of clinical problem solving. However, it may be difficult to recognise the logic and systematic construction of these schemes.

In most textbooks the amount of epidemiological information is rather limited. It is important to realise that such data are dependent on the clinical setting. The prevalence
of a certain diagnosis differs in a primary care setting, in a regional hospital and in a tertiary referral university medical centre. Therefore, it is easier and safer for medical students to use their preclinical knowledge in anatomy and pathophysiology and make a logical differential diagnosis according to this knowledge. With regard to the latter, the question is whether medical textbooks are structured in such a way that medical students can recognise the logic. If they can, this facilitates them to acquire this competence.

In other words: do medical textbooks teach in making a differential diagnosis and help to structure knowledge in a logical way?

METHODS

For four textbooks we made a comparison of the given differential diagnosis for four illustrative clinical problems. We gave special attention to the degree of logical categorisation. We compared one American comprehensive textbook (*Harrison*), two British rather concise textbooks (*Kumar, Souhami*) and one Dutch concise textbook (*Van der Meer*). Each of these books is widely used in medical schools in the Netherlands. In addition, we used the web-based version of *UpToDate*.

For four major clinical problems (upper gastrointestinal bleeding, anaemia, oedema and heart failure) we have summarised the presentation of the differential diagnosis in these textbooks in tables 1 to 4. The organising principle is listed and some examples are given. The information is gathered from the original text (sometimes with headings, or with bold or italic accents), from tables or figures and sometimes from a combination of these.

RESULTS

Upper gastrointestinal bleeding (table 1)

This symptom or sign can be analysed typically by an anatomy-based approach. Two textbooks use this approach. For each anatomical site some examples of lesions are given. *UpToDate* uses a pathophysiological approach, *Souhami* a combination of anatomical and pathophysiological. Two books use a nonspecific or epidemiological arrangement; this illustrates that such a listing is difficult to reproduce for inexperienced medical students.

Anaemia (table 2)

All textbooks adopt a pathophysiological approach, usually based on blood cell indices, with the addition of various examples. Only *van der Meer* uses a pathophysiological arrangement based on the mechanism; such a mechanistic approach is commonly used for cases with a shortage or deficit of cells or molecules: decreased production, increased destruction or increased loss. It is obvious that use of these mechanisms is logical, and perhaps therefore well known among teachers and students.

<table>
<thead>
<tr>
<th>TEXTBOOK</th>
<th>CATEGORIES</th>
<th>SPECIFIC ENTITIES</th>
<th>ORGANISING PRINCIPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harrison</td>
<td>Ulcers, varices, Mallory Weiss tears, erosions, erosive oesophagitis, malignancies</td>
<td>Epidemiological (with incidence rates)</td>
<td></td>
</tr>
<tr>
<td>Kumar</td>
<td>Oesophagus</td>
<td>Varices, etc.</td>
<td>Anatomic (with figure and incidence rates)</td>
</tr>
<tr>
<td></td>
<td>Stomach</td>
<td>Ulcer, etc.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duodenum</td>
<td>Ulcer, etc.</td>
<td></td>
</tr>
<tr>
<td>Souhami</td>
<td>Oesophagus</td>
<td>Carcinoma, etc.</td>
<td>a) Anatomic</td>
</tr>
<tr>
<td></td>
<td>Stomach</td>
<td>Ulcer, etc.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Duodenum</td>
<td>Ulcer, etc.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Systemic</td>
<td>Renal failure, clotting disorders, etc.</td>
<td>b) Pathophysiological</td>
</tr>
<tr>
<td></td>
<td>Vascular</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Swallowed blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van der Meer</td>
<td>Ulcer, varices, gastritis, gastric carcinoma, Mallory Weiss tears, oesophagitis</td>
<td>Nonspecific</td>
<td></td>
</tr>
<tr>
<td>UpToDate</td>
<td>Ulcerative or erosive</td>
<td></td>
<td>Pathophysiological</td>
</tr>
<tr>
<td></td>
<td>Portal hypertension</td>
<td>Varices</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vascular malformations</td>
<td>e.g. Angiomas, telangiectasias</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Traumatic</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Tumours</td>
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</table>
A useful pathophysiological approach to the differential diagnosis of oedema can only be found in UpToDate. The textbooks lack a logical differential diagnosis. They use different approaches (except van der Meer where there is no differential diagnosis of oedema), but none of them are systematic and therefore they are difficult to reproduce.

In all textbooks there is an extended review of the pathophysiology of oedema in certain circumstances, i.e. heart failure or hepatic cirrhosis.

**Heart failure (table 4)**

It is remarkable that for a rather difficult syndrome as heart failure, only two textbooks use a systemic approach. The organising principle is based on the pathophysiology, each book in a different way. It is obvious that the non-specific approach with a random list of various causes, as used in the three others, is not particularly helpful for medical students to make a differential diagnosis.

**DISCUSSION**

In this paper, we demonstrate how variable the presentation of differential diagnoses in medical textbooks is. Despite the availability of logically organised differential diagnoses, which are well known and widely used in teaching and clinical practice and easy to reproduce, it is disappointing that in respected textbooks an obvious systematic approach in differential diagnoses is often lacking. We compared only four textbooks and a web-based edition, and four clinical problems, but it is likely that other textbooks and additional differential diagnoses will yield similar results. It is remarkable that there seems to be no real difference between concise and comprehensive textbooks.

Clinical problem solving is difficult for students and even for their educators to teach it. Experienced clinicians often think associatively or by pattern recognition. They are familiar with the clinical presentation of diseases and are aware of the epidemiology in their own clinical setting. For students, lack of experience is a major handicap to understanding the clinical reasoning of experts and to memorising the differential diagnoses. Therefore, in modern medical curricula a systemic instruction of clinical problem solving is an essential part. Textbooks should be important aids in this learning process. Traditionally, however, they contain typical descriptions of diseases and these are insufficient for education in clinical problem solving. It is the world turned upside down for medical students.
down: the entry is the disease instead of the patient’s symptom or physical sign. Moreover, these descriptions apply to classical, full-blown diseases with the complete clinical picture that is only present in patients with advanced disease.\textsuperscript{11,12}

It is a positive point that more and more textbooks give attention to the approach to the patient and to differential diagnoses of clinical problems, but attention is rarely given to the principles of clinical problem solving. It is our experience that a systematic, logical set of differential diagnostic options can easily be remembered for similar cases in the future. Experienced clinicians may also use the systematic approach for complex cases when the diagnosis is not initially found.

A systematic approach is an aid in teaching clinical problem solving.\textsuperscript{1,2} It is remarkable that even in the literature on

<table>
<thead>
<tr>
<th>Table 3</th>
<th>The aetiology of oedema in various textbooks</th>
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<tbody>
<tr>
<td>TEXTBOOK</td>
<td>CATEGORIES</td>
</tr>
<tr>
<td>Harrison</td>
<td>Localised</td>
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<tr>
<td></td>
<td>Generalised</td>
</tr>
<tr>
<td>Kumar</td>
<td></td>
</tr>
<tr>
<td>Souhami</td>
<td></td>
</tr>
<tr>
<td>Van der Meer</td>
<td>Increased capillary hydraulic pressure</td>
</tr>
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<td></td>
<td>Hypoalbuminaemia</td>
</tr>
<tr>
<td></td>
<td>Increased capillary permeability</td>
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</table>

<table>
<thead>
<tr>
<th>Table 4</th>
<th>The aetiology of heart failure in various textbooks</th>
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</thead>
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<tr>
<td>TEXTBOOK</td>
<td>CATEGORIES</td>
</tr>
<tr>
<td>Harrison</td>
<td></td>
</tr>
<tr>
<td>Kumar</td>
<td>Myocardial dysfunction</td>
</tr>
<tr>
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<td>Volume overload</td>
</tr>
<tr>
<td></td>
<td>Obstruction to outflow</td>
</tr>
<tr>
<td></td>
<td>Obligatory high output</td>
</tr>
<tr>
<td></td>
<td>Compromised ventricular filling</td>
</tr>
<tr>
<td></td>
<td>Altered rhythm</td>
</tr>
<tr>
<td>Souhami</td>
<td></td>
</tr>
<tr>
<td>Van der Meer</td>
<td>Pressure overload</td>
</tr>
<tr>
<td></td>
<td>Volume overload</td>
</tr>
<tr>
<td></td>
<td>Inflow obstruction</td>
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<td>Myocardial dysfunction</td>
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<td>Coronary heart disease, hypertension, cardiomyopathy, valvular heart disease, pericardial disease, tachyarrhythmias, high output states</td>
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instruction of clinical problem solving only one article makes notice of this topic; it pleads for logical organisation of knowledge in medical textbooks. During the preparation of this manuscript a literature search in PubMed revealed no further hits on this subject since 1986. We conclude from our limited survey that it is hard for medical teachers giving instruction on systematic clinical problem solving to refer to textbooks. We feel this is a missed chance. Editors of textbooks should further improve their textbooks by paying attention to these systematic aspects. Textbooks with logical and systematic approaches to differential diagnosis will support and stimulate medical students and their teachers in the learning process of clinical problem solving.

Several medical journals review new editions of medical textbooks, and often compare them with the existing ones. We recommend reviewers to give more attention to these aspects of logical categorisation to help the present and future generation of clinicians.

ACKNOWLEDGEMENT

The contribution of I. Bakir in the initial part of the study is gratefully acknowledged.

REFERENCES

More on bleomycin and scuba diving

The article ‘Bleomycin and scuba diving: to dive or not to dive?’, by G. Huls and D. ten Bokkel Huinink in the Netherlands Journal of Medicine considers two potential problems regarding oxygen exposure in the post-bleomycin patient. The problems concern anaesthesia and the scuba diver. This is important in view of a recent review about the long-term medical care of survivors of testicular cancer that did not consider either of these issues. Furthermore, these issues are critically important to the primary care physician, who may be:
- the physician of record for one of these patients;
- the consultant to clear the patient for surgery or for scuba diving;
- the first responder to evaluate and treat one of these patients after a diving-related accident.

In the surgical case, the risk is not always obvious because the operative procedures may not be related to the patient’s malignancy (for example appendectomy, hernia repair and trauma). In the scuba diving case, the risks may not be recognised. Nevertheless, all authors do not agree that high supplemental oxygen contributes to morbidity and mortality in the post-bleomycin patient.

The commentary below adds to the arguments of Huls and Bokkel Huinink and is applicable to other oncology patients (such as those with Hodgkin’s disease and non-Hodgkin’s lymphoma), who may consider scuba diving post-bleomycin therapy.

Scuba diving is a growing recreational sport; new scuba divers are certified each year worldwide and, as with testicular cancer patients, many are young men. Diving may be particularly hazardous for post-bleomycin patients not only because of increased risk of oxygen toxicity in their lungs due to high oxygen partial pressure, but also because of barotrauma and complications from standard treatment for scuba diving-related barotrauma and decompression illness.

First, as described by Huls and Bokkel Huinink, the partial pressure of inspired oxygen is a function of the depth of the dive. When a scuba diver breathes compressed air (21% oxygen at the surface) at a depth of 29.7 meters (approx. 90 ft depth) of seawater, the partial pressure is 0.84 atmosphere or the equivalent to breathing 84% oxygen on the surface. Oxygen toxicity in normal divers is limited by the short duration of exposure at depth due to the risk of decompression illness. Based on the anaesthesia experience, prior bleomycin exposure may increase the risk of oxygen-exacerbated complications in the scuba diver. Also, in the post-bleomycin scuba diver, the complications of oxygen toxicity may be more severe with oxygen-enriched mixtures also used in diving, popularly known as Nitrox (‘nitrox’: 60% nitrogen and 40% oxygen is a typical example). Second, bleomycin may induce clinical and subclinical pulmonary fibrosis in as many as 30% of patients who receive the drug. The damaged, less distensible lungs place the diver at risk for barotrauma (such as pneumothorax, arterial gas embolism and pneumomediastinum). Third, the treatment of a diver with barotrauma and decompression illness includes 100% oxygen. As described for anaesthesia and the scuba diver at depth, high supplemental oxygen as treatment may result in severe morbidity and mortality in the post-bleomycin patient.

At this time, in a recreational setting, there are no data to support recommendations for safe oxygen exposures, minimum diving depths, and length of time at depth for the potential post-bleomycin scuba diver. Pending adequate data to permit safe scuba diving for these individuals, the post-bleomycin patient should be cautioned about the potential risk of serious morbidity and mortality with scuba diving and with the treatment of at least two scuba diving complications (decompression illness and barotrauma). Until there is further data available about the safety of high oxygen partial pressure exposure, for the man cured of testicular cancer with a bleomycin-containing regimen, a strong warning not to dive may not be too conservative.

R.M. White
12054 Eaglewood Court, Silver Spring, MD 20902, USA, e-mail: rmwhite@wesleyan.edu
REFERENCES


REACTION FROM THE AUTHORS

In response to the ‘letter to the editor’ from Dr R.M. White, we agree that the increased risk of barotrauma in the damaged lung (by bleomycin) and the potential risk of high oxygen treatment for barotrauma and decompression illness strengthen our discouragement about diving after bleomycin-containing chemotherapy.

G. Huls
D. ten Bokkel Huinink

ANSWERS TO PHOTO QUIZ (ON PAGE 370)
A PATIENT WITH PANCYTOPENIA AND MICROCYTIC MEGALOBLASTIC ANAEMIA

DIAGNOSIS

This woman has a combined deficiency of vitamin B12 and iron due to pernicious anaemia. The Schilling test later appeared to contain a nonfunctioning intrinsic factor probe. After suppletion of both cobalamine and iron her blood levels normalised and the symptoms disappeared.
Surgery in pictures

A.J.P.M. Overbeke

Executive editor Nederlands Tijdschrift voor Geneeskunde,
PO Box 75791, 1070 AZ Amsterdam

In 2002, the Association of Surgeons of the Netherlands celebrated its centenary. And in keeping with such a jubilee, the board of this scientific society decided that a commemorative volume should be published. The history of surgery in the Netherlands had already been described on two previous occasions in the association’s history, in 1977 by Kuijjer and in 1987 by De Moulin. As the two previous books were predominantly written records in which the illustrations merely enlightened the text, the editors decided that this commemorative volume should primarily be an illustrative history. As was to be expected the result was splendid: a significant part of surgery is about portraying diseases that are then restored, removed or replaced. And both the methods and those performing these can be depicted.

Surgery in pictures consists of two volumes: Pictures from the history of Dutch surgery and Surgery portrayed in Dutch works of art. The richly illustrated first volume not only describes the achievements within the various branches of surgery, but also the development of operations for different organs, including the abdominal organs, bones, lungs and vessels. Foreign influences on Dutch surgery and the role of women in surgery are also covered. The second volume illustrates more than one hundred works of art from Dutch collections that portray the history of surgery and the history of art portraying surgery. This part of art history covers the period from before 1600 right up to the present day and includes paintings, drawings, sculptures, graphics and sketches.

The two books complement each other superbly and are a feast for the eye. Of course it was never the intention to produce a definitive work, as this would have been an impossible task, and neither is it a strictly scientific approach to the history of surgery in the Netherlands, which in any case was not necessary. The editors and all those who contributed to this memorable work can be justifiably pleased with the result. Nonsurgical colleagues will also enjoy browsing though this book and reading the passages of interest to them.

Yet this is easier said then done. Surgery in pictures cannot be obtained from a bookshop. However, it is definitely worthwhile dropping in on a surgeon you know to ask if you can take a look or indeed borrow it, to get an idea of the rich and also in part well-known history of Dutch surgery. For those with a genuine interest, a copy can be obtained via the secretariat of the Dutch Surgical Society. So this commemorative volume not only describes the past but it also creates new links or strengthens existing links between the different medical professions.

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<th>Title</th>
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<td>A. van der Tol, J. Keeman</td>
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<td>Publisher</td>
<td>Six Art Promotions bv, Amsterdam, the Netherlands</td>
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Aims and scope
The Netherlands Journal of Medicine publishes papers in all relevant fields of internal medicine. In addition to reports of original clinical and experimental studies, reviews on topics of interest or importance, case reports, book reviews and letters to the Editor are welcomed.

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

Declaration
It is the author’s responsibility to seek permission from the person or party concerned for the use of previously published material, such as tables and figures. In addition, persons who are recognisable on photographs must have given permission for the use of these.

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

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

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

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

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

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

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

The Results should be presented precisely without discussion.

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

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

Examples:


Please note that the first six authors should be listed; when seven or more, list only the first three and add et al.

Do not include references to personal communications, unpublished data or manuscripts either ‘in preparation’ or ‘submitted for publication’. If essential, such material may be incorporated into the appropriate place in the text. Recheck references in the text against reference list after your manuscript has been revised.

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Brief reports containing concise reports on original work will be considered for publication. Case reports which are relevant for understanding the pathophysiology or clinical presentation of disease may also be accepted under this heading. Articles published in this section should be no longer than 1000 words, and be supplied with a summary of about 60 words, preferably no more than two figures and/or tables, and no more than 15 references.

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