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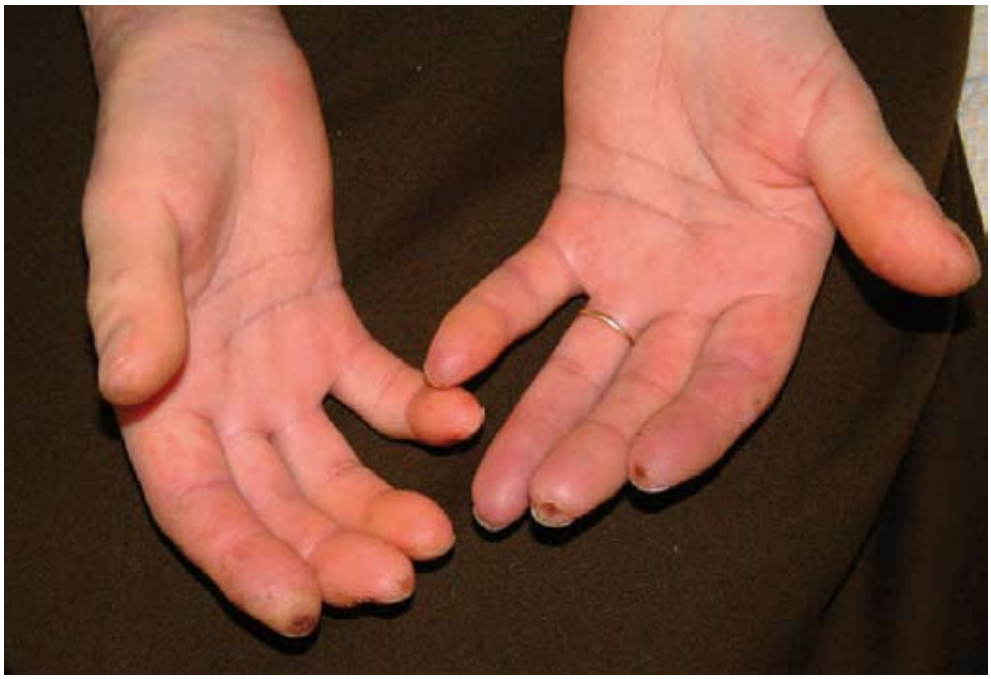

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Alemtuzumab in treatment of CLL

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Budesonide in Crohn's disease

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VAN ZUIDEN COMMUNICATIONS

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EDITORIAL

Budesonide: a useful tool in the maintenance treatment of Crohn's disease?

E.J. Schoon

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Maintaining remission over a long period of time is a great challenge in the management of Crohn's disease. For a long-time corticosteroids were the mainstay of pharmacological treatment of active Crohn's disease. In fact, corticosteroids have been used in inflammatory bowel disease (IBD) rather successfully as induction therapy since the 1950s.[1] However, for maintenance treatment this class of drugs is not considered to be very effective. Although some 80% of IBD patients respond to corticosteroids, there is a considerable loss of efficacy on maintenance treatment, which might be explained by the selection of steroid-resistant populations of lymphocytes in the gut mucosa. Some patients are unable to stop taking corticosteroids because of loss of well-being and risk for flares, which might be explained by withdrawal symptoms and other effects on the central nervous system. The major disadvantage of long-term maintenance treatment is the side effects related to corticosteroids, the worst being irreversible osteoporosis eventually leading to vertebral fractures.[2]

The topically active synthetic steroid budesonide has far fewer side effects because of a high first pass effect in the liver after oral administration, and is therefore considered to be safer than prednisolone. When it was introduced, clinicians hoped that besides effective induction therapy, which will not be discussed in this editorial, they would have access to a new tool for safe and effective long-term treatment.

In a large multicentre international study focused on budesonide treatment in Crohn's disease from our group, we found it hard to recruit Dutch patients on longterm steroid treatment.[3] This was probably due to the extended use of immunosuppressants such as azathioprine and methotrexate in the last decade of the last century. These drugs are particularly effective for long-term treatment and only a small group of IBD patients are intolerant to both. Methotrexate is more effective than placebo in maintenance, but the results are less convincing than with azathioprine. Azathioprine has been shown to decrease the relapse rate at one year from 40% on placebo to 5 to 10%.

The introduction of anti-TNF-α inhibitors led to other perspectives for long-term treatment of Crohn's disease. These drugs appear to be very effective in the maintenance treatment of Crohn's disease. As with every medical treatment, there have been concerns about loss of efficacy, side effects and safety.[4] In the light of these developments, the role of corticosteroids is changing. Currently, the conservative pharmacological step-up approach is shifting towards a top-down or a more rapid step-up approach as patients with a shorter disease history and younger patients tend to respond better and longer.[5] Treatment of Crohn's disease in this aspect is comparable with treatment of rheumatoid arthritis patients.

In this issue De Jong *et al.* describe an impressive, large multicentre study conducted both in the Netherlands and Germany.[6] A total of 160 Crohn's disease patients in remission were included and were randomised to a maintenance regimen of 6 or 9 mg/day of budesonide controlled-release (Budenofalk®). There was no difference in the one-year relapse rate, and the time to relapse was similar. However, the one-year relapse rates were very low (24 *vs* 19%).

Are the results of this study surprising? In the predetermined pooled analysis of four randomised controlled trials with budesonide 3 or 6 mg *vs* placebo in patients with medically induced remission, time to relapse was prolonged but without a difference in one-year relapse rate![7] Thus, it would have been surprising if budesonide 9 mg were to have been more effective than 6 mg for the long-term relapse rate after one year.

In addition, low-dose oral budesonide cannot be recommended for the prevention of postoperative relapse in Crohn's disease.[8] Oral budesonide, 6 mg/day, offered no benefit in prevention of endoscopic recurrence after surgery for ileal/ileocaecal fibrostenotic Crohn's disease

but decreased the recurrence rate in patients who had undergone surgery for disease activity.[9]

What is the clinical impact of the results? This study confirms that budesonide is equally potent in prolonging the time remission in both a 6 or 9 mg/day dose. This study demonstrates that a 9 mg dose is no better than 6 mg for this purpose. In the study by de Jong *et al*. a low one-year relapse rate was found, most likely due to an inclusion of patients whose Crohn's disease followed a relatively mild course. In view of these findings, it is unlikely that new large placebo-controlled studies with budesonide controlled-release will ever be performed. Presently, the number of potential new drugs for IBD exceeds the number patients available for these studies.

In patients with mild to moderate ileocaecal Crohn's disease, budesonide controlled-release is effective for remission induction therapy but the effect of maintenance therapy after remission is medically achieved in doses of up to 9 mg daily is in my opinion doubtful.

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REVIEW

Familial Mediterranean fever: clinical, molecular and management advancements

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ABSTRACT

Familial Mediterranean fever (FMF), the most frequent of the periodic fever syndromes, is an autosomal recessive disease, predominantly affecting people of Mediterranean descent. The disease is caused by mutations in the MEFV gene, encoding the pyrin protein thought to be associated with the interleukin-1 related inflammation cascade. The condition manifests as attacks of serositis, commonly involving the abdomen, chest or joints, typically accompanied by fever and elevated acute phase reactants. Attacks subside spontaneously within one to three days, without residue. Continuous treatment with colchicine, at a daily dose of 1 to 2 mg, reduces attack frequency, duration and intensity in the majority of patients, and also prevents the development of secondary amyloidosis, the most dreaded complication of the disease. In this communication we review the current state of the art in the diagnosis and care of FMF patients, starting with the presentation of a typical case.

KEYWORDS

Diagnosis, familial Mediterranean fever, treatment

CASE REPORT

An 18-year-old male of Turkish descent was referred to the clinic for evaluation of recurrent abdominal pain and fever. He had recently undergone an appendectomy, during an attack of severe abdominal pain, accompanied by fever of 38.5°C and signs of peritoneal inflammation, localised to the right lower quadrant. Analysis of blood, drawn prior to surgery, revealed a leucocytosis of 15,000/μl with a left shift, normal platelet count and haemoglobin level. Blood chemistry was normal, except for an elevated fibrinogen (680 mg/dl, normal<400). Erythrocyte sedimentation rate was high (60 mm/h) as was the C-reactive protein (CRP; 15 mg/dl, normal <0.5). There was trace of protein on urinalysis. Given the clinical and laboratory findings, compatible with acute appendicitis, the surgeons were surprised when only a nonphlegmonous appendix, with minimal serosal irritation, and small amount of turbid peritoneal fluid were found on laparotomy. Their bewilderment increased when, after an uneventful recovery, the patient returned to the emergency room with the same clinical findings a month later. A thorough anamnesis at that time yielded the information that he has suffered from similar episodes, about twice yearly, since the age of 15. The episodes resolve within a day or two, and are alleviated by a cocktail of an NSAID and acetaminophen. He knows that abdominal pain 'runs in the family', as his father and two of his cousins suffer from similar episodes, as did an aunt who had later died of end-stage renal disease. Once attacks resolve, he feels completely well, he is an avid sportsman and experiences no limitations in his pursuits.

EPIDEMIOLOGY AND GENETICS

Familial Mediterranean fever (FMF), an autosomal recessive condition, affects more than 100,000 people worldwide, and as such, is the most common of the hereditary periodic fevers. The disease is most prevalent among non-Ashkenazi Jews, Arabs, Turks and Armenians, with carrier frequencies of 1:5 to 1:16, 1:5, 1:5, and 1:7, respectively. Yet, it is observed worldwide due to the extensive population movements of the 20th century.[1]

FMF is caused by mutations in the MEFV gene, which encodes for the pyrin protein, expressed primarily in the myeloid cell lineage. Pyrin belongs to a class of proteins involved in the regulation of apoptosis and inflammation.

Its N-terminal pyrin domain interacts with the ASC adaptor protein, regulating caspase-1 activation and consequently, IL-1β production.[2] Mutations interfere with the role of the pyrin domain, allowing an uninterrupted inflammatory cascade.

CLINICAL FEATURES

Abdominal pain is the most common presenting feature of FMF, eventually occurring in 95% of patients.[3] The abdominal pain may be diffuse or localised, and varies in intensity from mild, without overt signs of peritonitis, to the more typical severe pain, which necessitates bed rest. It is exacerbated by breathing and accompanied by signs of peritoneal inflammation. Attacks begin suddenly, usually without a recognisable precipitating event and last for 6 to 96 hours. Women of child-bearing age may note a tendency for attacks to occur around menstruation.

Arthritis, typically involving a single knee or ankle, is another major disease manifestation, associated with local redness, swelling and tenderness.[3] Acute arthritic attacks tend to subside within the same time frame as described for abdominal attacks. Arthritis of upper extremity joints, protracted arthritis and a seronegative spondyloarthropathy have all been reported in association with FMF, in a small percentage of patients.[4,5]

Chest attacks, due to inflammation of the pleura, are reported by 30% of the patients. Usually they are unilateral and similarly to abdominal and joint attacks they resolve within hours to several days.[3]

A fourth disease feature, erysipeloid erythema, is described in 7 to 40% of patients.[3] It consists of erysipelas-like erythematous, shiny plaques, typically appearing on the shins.

FMF attacks are commonly accompanied by fever, often as high as 39°C, although not universally present in all patients or during all attacks in the same patient. Fever may at times be the only manifestation of an attack of FMF. Patients may describe recurring episodes of fever, without an obvious underlying cause or painful manifestation, each lasting a day or two, and resolving spontaneously.

Rare but distinctive attack manifestations of FMF include pericarditis (<1% of patients), acute orchitis (<5% of male patients) and protracted febrile myalgia (a steroid responsive condition, associated with paralysing muscle pain in the extremities, tenderness and, at times, a vasculitic rash and nephritis), entities which should be routinely enquired about when attempting to establish the diagnosis.

More than 50% of FMF patients experience premonitory symptoms, or a prodrome, heralding the FMF attack.[6] The prodrome may include discomfort at the impending attack site or various constitutional, emotional, and physical complaints, including irritability, dizziness, increased appetite, and altered taste sensation. A prodrome portends an attack in almost 100% of occurrences in affected patients and is thus a valid sign of an imminent attack. This time interval, which lasts 12 to 24 hours, may be used for prompt institution of preventive therapy prior to the impending spell, such as interferon-α (IFN-α).[7]

FMF patients markedly differ with respect to the features of their disease. The frequency of attacks, for example, may vary from several times a month to once every few years. The duration of the attacks ranges from several hours to several days. The site involved and the intensity in each site may also differ between patients and in the same patient in different attacks during the course of the disease.

Some patients may experience chronic manifestations in addition to the typical paroxysmal disease course. These may include features of spondyloarthropathy,[5] with pain and inflammation of central joints, fibromyalgia,[8] with diffuse bone, muscle and joint pain, as well as severe leg and/or foot pain on exertion,[9] sometimes associated with ankle swelling. An enlarged spleen, anaemia of chronic disease, and continuously elevated acute phase reactants may be found in 30% of inadequately treated patients.[10,11]

For the purpose of comparing patients, each exhibiting a diverse clinical spectrum, and in order to estimate disease burden, a severity scale was established. The scale is adjusted for two patient situations: colchicine-naive patients, usually in the process of undergoing a diagnostic evaluation, and colchicine-treated patients, under optimal control (*table 1*).[12]

LABORATORY ANALYSIS

Acute FMF attacks are associated with a nonspecific increase in inflammatory mediators, such as serum amyloid A, fibrinogen, ESR and CRP, as well as an elevation of the white blood cell count, typically returning to baseline levels in between attacks.[13-15] Urinalysis may detect haematuria and/or proteinuria, the significance of which is discussed subsequently. As mentioned previously, chronic subclinical inflammation, manifested by elevated CRP and serum amyloid A protein (SAA) levels during clinical quiescence, may be found in 30% of patients.[10]

Over the years, a myriad of cytokines, chemokines and other inflammation-associated proteins have been studied in FMF patients. These include IL-1, 4, 5, 6, 10, 12, 18 as well as TNF-α and γ, cytokine-associated receptors, complement proteins, adhesion molecules, growth factors, immunoglobulins and a large spectrum of antibodies.[11,16-26] The overall picture that emerges is that FMF is not an autoimmune disease. Rather, the cytokine/chemokine pattern is consistent with nonspecific inflammation. Unfortunately, these studies have not yielded FMF disease-specific or diagnostic laboratory tests.

Table 1. *Familial Mediterranean fever disease severity score*

Subgroup	Severity	Number of features	Features
Patients either not yet taking colchicine or not responding to colchicine therapy	Severe	2 or more	1. ≥2 attacks per month 2. Involvement of more than 1 attack site in >25% of attacks 3. Involvement of more than 2 attack sites during the disease course
	Moderate	1 or more	1. 18-24 attacks/year 2. Attack duration ≥4 days, on most attacks
	Mild		Neither severe nor moderate disease
Patients under optimal colchicine therapy*	Severe	3 or more	1. Involvement of more than 1 attack site in >25% of attacks 2. Involvement of more than 2 attack sites during the disease course 3. ≥2 mg/d colchicine (or less if intolerant) 4. ≥2 pleuritic attacks during disease course 5. ≥2 erysipeloid-erythema attacks during disease course 6. Age of onset ≤10 years
	Moderate	2	
	Mild	0-1	

*Attack-related features (e.g. frequency, site affected, etc.) refer to disease activity prior to colchicine treatment.

GENETIC ANALYSIS

Although over 80 mutations in the MEFV gene have been described, the majority of cases are caused by four mutations clustered on a single exon: M694V, V726A, M680I and M694I, the prevalence of which varies according to the population studied. In Turks, who constitute the largest ethnic group with FMF in the Netherlands, M694V would, in all probability, be the leading MEFV mutation (51% according to the Turkish FMF study group) followed by M680I (14%) and V726A (9%). Overall, around 80% of FMF patients have an identifiable MEFV mutation; 57% have two mutations, 26% have a single mutation, while 16% have no identifiable mutation.[27,28] A comparable distribution is found in all ethnic groups in which FMF is prevalent. Currently, the underlying mechanism for the expression of FMF in heterozygous or MEFV mutation-free patients is unclear.

The role of the exon 2, E148Q mutation, as a disease-causing mutation is controversial. This non-founder mutation is found in populations in which FMF is distinctly rare, such as the Japanese,[29] Chinese and Punjabi Indians.[30] Additionally, E148Q homozygotes are rarely found in the FMF population.[27] Yet, despite the slim penetrance of this mutation, most FMF experts refer to it as a mild disease-causing mutation. This mutation appears in 3 to 18% of the major ethnicities which are at risk for FMF.[27,31,32]

DIAGNOSTIC CRITERIA

Despite the cloning of the MEFV gene and the ongoing identification of new mutations, the diagnosis of FMF remains a clinical one for several reasons, the most notable of which is mentioned above, namely, the lack of identifiable MEFV mutations in at least 20% of patients with clinically proven FMF. The importance of making the diagnosis of FMF in patients without identifiable mutations is underscored by the observation that when M694V homozygotes, who tend to suffer a more severe disease course, are excluded, patients who bear no MEFV mutations are phenotypically similar to patients with recognisable mutations. The Tel Hashomer criteria (*table 2*) form the basis of the clinical diagnosis.[33] Clinical criteria are combined with results of MEFV mutation analysis and a therapeutic trial,[34] monitored by clinical response and SAA levels,[35] in a diagnostic algorithm (*figure 1*), which goes through the information and investigations, by the order in which they are usually processed in an office visit of a new patient suspected of having FMF.

THERAPY

Colchicine, an alkaloid, originally extracted from plants of the genus *Colchicum*, has been used in the treatment of gout since the first century CE. In 1972, Goldfinger first described its effectiveness in preventing FMF attacks, by reporting his experience with colchicine in five patients.[36] The use of colchicine in FMF over the past three decades has dramatically changed the course of the disease in two aspects. First, a daily dose of 1 to 2 mg renders most patients asymptomatic, and in others, reduces attack frequency and/or duration. Only 10% of patients are colchicine resistant and continue to suffer attacks at the same intensity as before. Second, reactive amyloidosis, the most feared complication of FMF (discussed subsequently), is almost completely prevented in patients adherent to their colchicine therapy, including the 10% of patients who fail colchicine attack prophylaxis.

The initial colchicine dose in patients with normal renal function and without proteinuria is 1 mg/day. If patients continue to suffer frequent attacks, the dose can be increased by 0.5 mg/day up to a total of 2 mg/day. For compliance purposes, we suggest taking up to 1.5 mg in a single, consolidated daily dose.

Table 2. *Criteria for the diagnosis of familial Mediterranean fever*[*]

Major criteria

Typical attacks

1. Peritonitis (generalised)
2. Pleuritis (unilateral) or pericarditis
3. Monoarthritis (hip, knee, ankle)

Minor criteria

1-3. Incomplete attacks involving 1 or more of the following sites:
   1. Abdomen
   2. Chest
   3. Joint
4. Exertional leg pain
5. Favourable response to colchicine

Supportive criteria

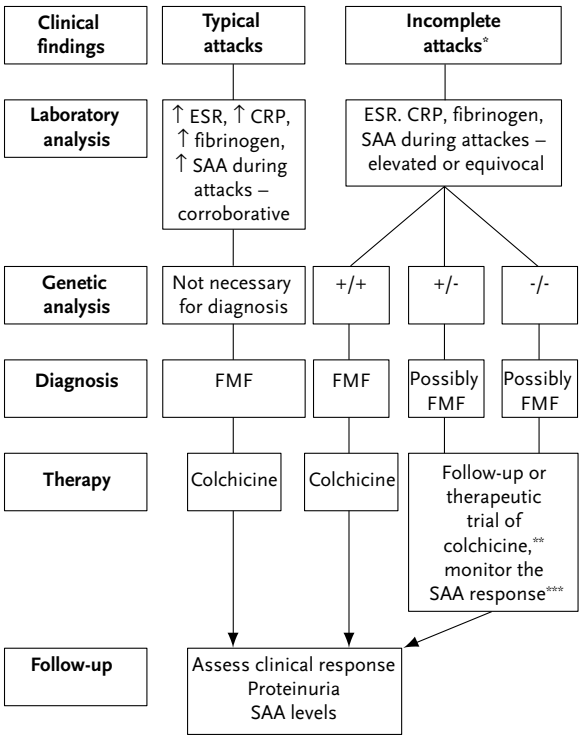
1. Family history of familial Mediterranean fever
2. Appropriate ethnic origin
3. Age <20 years at disease onset

4-7. Features of attacks
   4. Severe, requiring bed rest
   5. Spontaneous remission
   6. Symptom-free interval
   7. ransient inflammatory response, with 1 or more abnormal test result(s) for white blood cell count, erythrocyte sedimentation rate, serum amyloid A, and/or fibrinogen

8. Episodic proteinuria/haematuria
9. Unproductive laparotomy or removal of 'white' appendix
10. Cosanguinity of parents

[*]**The requirements for diagnosis of familial Mediterranean fever are ≥1 major criteria, or ≥2 minor criteria, or 1 minor plus ≥5 supportive criteria, or 1 minor criterion plus ≥4 of the first 5 supportive criteria. Typical attacks are defined as recurrent (≥3 of the same type), febrile (rectal temperature of 38°C or higher), and short (lasting between 12 hours and 3 days). Incomplete attacks are defined as painful and recurrent attacks that differ from typical attacks in 1 or 2 features, as follows; 1) the temperature is normal or lower than 38°C; 2) the attacks are longer or shorter than specified (but no shorter than 6 hours or longer than a week; 3) no signs of peritonitis are recorded during the abdominal attacks; 4) the abdominal attacks are localised; 5) the arthritis is in joints other than those specified. Attacks are not counted if they do not fit the definition of either typical or incomplete attacks.**

Figure 1. *Diagnostic algorithm*



ESR = erythrocyte sedementation rate; CRP = C-reactive protein; SAA = serum amyloid A protein; FMF = familial Mediterranean fever.
[*]**Typical and incomplete attacks are defined in *table 1*.**
[**]**Therapeutic trial with colchicine denotes evaluation of patient response to colchicine. If attacks continue, the colchicine dose is increased gradually to 2 mg/day. If attacks subside, colchicine is discontinued. Resumption of attacks serves to validate the diagnosis of FMF.** [***]**SAA levels during remission and their response to colchicine administration.**

The most frequent side effects of colchicine therapy are gastrointestinal: cramp, abdominal pain and diarrhoea. These may be avoided by commencing treatment at a subtherapeutic dose of 0.5 mg/day, and gradually increasing the daily dose by 0.5 mg increments, in divided daily doses. In more difficult cases, oral desensitisation, similar to that used in cases of allergic reactions, may be attempted. An optional desensitisation protocol is as follows: 1 ml of a 1 mg ampoule of colchicine diluted in 1000 ml of 5% glucose is given initially, after which the dose is doubled daily, until 0.25 mg is tolerated, at which point an oral tablet is commenced.[37] Most patients, however, tolerate an initial dose of 1 mg/day without perturbations.

As previously mentioned, the majority of patients experience a prodromal phase prior to the full blown FMF attack.[6] Institution of preventive therapy during this time interval may curtail the impending attack. Therapeutic options include a transient increase in the oral colchicine dose or the addition of a nonsteroidal anti-inflammatory drug (NSAID). If these simple measures fail, administration of IFN-α may be attempted. Although not studied in the prodromal phase proper, IFN-α was found to shorten attack duration and result in a depressed inflammatory response in some patients when administered in the early stages of the attack.[7]

TREATMENT OF RESISTANT CASES

About 10% of FMF patients suffer frequent attacks, despite compliance with colchicine therapy and are therefore deemed nonresponders. Reasons for nonresponsiveness remain a puzzle. Yet, an abnormality in colchicine consumption by mononuclear cells suggests that these patients sustain an additional genetic defect.[38] Therapeutic options for this important group of patients are unsatisfactory as proposed agents have only been studied in individual cases or in small, nonrandomised

trials. Nonetheless, patients suffering frequent or disabling attacks, on a maximal tolerated dose of oral colchicine, may be offered a therapeutic trial with 1 mg weekly intravenous colchicine, in addition to the regular oral regime.[39] Alternatively, efficacy of TNF inhibitors has been shown in several case reports, with significant improvement in attack parameters for both etanercept and infliximab.[40-42] Thalidomide, an anti-inflammatory agent with anti-TNF properties, was also efficacious in a small group of patients, albeit, disquieting due to side effects such as peripheral neuropathy and teratogenicity.[43] A fourth option might be IFN-α, which was successful at halting 18/21 FMF attacks in an open label trial,[44] although results were less encouraging in a larger placebo-controlled trial in which it was no more efficacious than placebo.[7]

REACTIVE AMYLOIDOSIS

Reactive (or secondary) AA amyloidosis is the most devastating complication of FMF. It is caused by the extracellular deposition of amyloid fibrils, consisting of β-pleated sheet configured polymers of the N-terminal fragments of the acute phase protein, SAA.[45] As amyloid slowly accumulates in various organs and tissues, organ dysfunction ensues, most prominently in the kidneys. The most common and perhaps the exclusive presenting feature of renal involvement due to AA amyloidosis is proteinuria, gradually progressing into nephrotic syndrome and/or renal dysfunction. Before the advent of colchicine therapy, amyloidosis was reported to occur in about 75% of FMF patients over the age of 40 years.[3] Ethnicity affects the prevalence of amyloidosis, with a high prevalence found in untreated North African Jews, Armenians and Turks. The explanation may reside in the increased prevalence of the M694V mutation in these ethnicities, a mutation that has also been generally recognised as a risk factor for development of amyloidosis in most ethnic origins other than Turks,[46,47] Additional factors which may increase the risk for developing amyloidosis are the SAA1 α/α genotype, male gender, joint attacks and a positive family history of amyloidosis.[48,49]

As proteinuria is the earliest sign of amyloidosis in FMF, patients should have a general urinalysis bi-yearly. If persistent proteinuria is revealed, the colchicine dose should be increased to 2 mg/day and histological confirmation obtained, by rectal (which is easier to attain and may show involvement prior to clinical symptoms) or by renal biopsy. Should proteinuria progress despite colchicine dose escalation, a therapeutic trial with the investigational compound eprosidate,[50] or with an anti-TNF preparation,[40-42] should be considered. Implication of TNF antagonists in FMF amyloidosis is inferred from case reports and retrospective analysis showing regression of proteinuria in non-FMF related reactive amyloidosis.[51] Also, angiotensin-converting enzyme inhibitors and cholesterol-lowering statins are typically added to the therapeutic regime, although their benefit in this patient population has not been evaluated. FMF patients with end-stage renal disease requiring dialysis therapy or renal transplantation should continue receiving colchicine, 2 mg/day, or less, if suffering from diarrhoea, in order to mitigate attacks and prevent amyloidosis in other organ systems as well as recurrent graft amyloidosis in the latter group.[52]

DIFFERENTIAL DIAGNOSIS

To the astute clinician, the diagnosis of FMF is generally straightforward, based on clinical criteria of recurrent attacks at typical sites. However, the episodic nature of the disease as well as its clinical manifestations, which mimic a myriad of disease states (*table 3*), coupled with, unfortunately, the lack of awareness of FMF on the part of physicians, contribute to the average diagnostic delay

Table 3. *Differential diagnosis of familial Mediterranean fever*

Abdominal attacks (recurrent peritonitis)
Appendicitis
Diverticulitis
Cholecystitis
Pyelonephritis
Pelvic inflammatory disease
Pancreatitis
Recurrent abdominal attacks (without peritonitis)
Peptic disease
Renal colic
Endometriosis
Menstruation pain
Irritable bowel syndrome
Chest attacks (recurrent pleuritic chest pain)
Pulmonary embolism
Pleuritis (idiopathic, infectious, autoimmune)
Pericarditis (idiopathic, infectious, autoimmune)
Joint attacks (recurrent synovitis)
Gout
Pseudogout
Spondyloarthropathy
Juvenile idiopathic arthritis
Febrile attacks (recurrent)
Lymphoma
Infections (malaria, relapsing fever)
PFAPA (periodic fever, aphtous stomatitis, pharyngitis, adenopathy)
Systemic conditions (recurrent febrile attacks involving ≥2 systems)
Inflammatory bowel disease
Hyper IgD syndrome
TNF receptor associated periodic syndrome
Acute intermittent porphyria
Behçet's disease
Systemic lupus erythematosus
Adult Still's disease

of ten years.[53] Apart from regional and systemic diseases, detailed in *table 3*, hyperimmunoglobulinaemia D and periodic fever syndrome (HIDS) and tumour necrosis factor receptor-associated periodic syndrome (TRAPS), deserve special mention. These diseases, together with FMF and the rare cryopyrin-associated periodic syndromes (CAPS), constitute the bulk of hereditary periodic fevers.

TRAPS is a dominantly inherited disorder, caused by mutations in the TNF receptor and characterised by recurrent attacks of abdominal pain associated with disabling myalgias, erythematous migratory rash, conjunctivitis and periorbital oedema.[54,55] The disease was originally described in patients of Irish/Scottish ancestry but was later identified in other ethnicities worldwide. The age of onset, abdominal involvement, rash, myalgias and propensity for amyloidosis resemble FMF. However, TRAPS attacks tend to be longer than FMF attacks (up to several weeks as opposed to an average of three days in FMF). Myalgia and localised, painful erythema, which are the hallmark of TRAPS, are less common in FMF and migration of the rash and eye involvement are not features of the latter.

HIDS is an autosomal recessive disease, caused by mutations in mevalonate kinase.[56] It is mainly found in patients of Dutch and Western European origin, and should be differentiated from FMF, especially in these populations. HIDS typically appears in the first year of life with attacks consisting of spiking fever accompanied by abdominal pain, cervical lymphadenopathy, hepatosplenomegaly, arthralgias and skin rash. Attacks tend to dissipate within four to six days only to recur periodically in intervals of several weeks.[57] While hepatosplenomegaly may be found in FMF, peripheral lymphadenopathy, diffuse rash and oligo-polyarthritis are not attributes of the disease. Moreover, HIDS is associated with a marked elevation of serum immunoglobulin D and is only rarely (less than 1%) complicated by amyloidosis, further distinguishing the syndrome from FMF.

CASE INTERPRETATION AND CONCLUSION

Our case depicts a young adult with short episodes of severe abdominal pain, associated with fever, which resolve spontaneously, only to recur after a variable asymptomatic period. Peritonitis as the underlying cause of the clinical picture was observed in the episode ending in appendectomy, which was comparable with the other attacks. This scenario in itself fulfils the criteria for the diagnosis of FMF. His age, ethnic origin, history of explorative laparotomy and family history are corroborative details which strengthen the diagnosis in the present case and serve to affirm it in atypical presentations. Also, FMF attacks are typically associated with an inflammatory response, as depicted. Genetic analysis was not given, intentionally, as it is noncontributory in a typical case, such as this one. Moreover, even with negative results on mutation analysis, the diagnosis of FMF is definite in this case. However, in atypical presentations, homozygosity on mutation analysis serves to confirm the diagnosis, while heterozygosity typically warrants a therapeutic trial with colchicine. Therapy with colchicine serves a dual purpose of ameliorating attack frequency and severity as well as preventing secondary amyloidosis.

Of note, the frequency of appendectomy in FMF patients is double the reported rate in the general population (40% *vs* 12 to 25%), while the rate of noninflamed appendectomies is extremely high (up to 80%), undoubtedly due to the overlapping clinical presentation of the two diseases (unpublished observation). Reliance on clinical parameters, namely a change from the regular diffuse involvement to right lower quadrant abdominal pain, has been shown to be the best predictor of an inflamed appendix in FMF patients. Had the history of previous attacks, not dissimilar from the index one, been elicited from the patient, the unnecessary appendectomy could have been avoided.

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REVIEW

Water in health and disease: new aspects of disturbances in water metabolism

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ABSTRACT

Vasopressin is a critical regulator of water homeostasis. There are two major receptors for vasopressin: V1 and V2 receptors. Disturbances in water balance are commonly encountered in clinical practice and can be divided into disorders of urinary dilution and concentration. The major representatives of such disorders are diabetes insipidus and the syndrome of inappropriate secretion of antidiuretic hormone (SIADH).

Recent studies show that genetic forms of nephrogenic diabetes insipidus are due to mutations in the genes coding for the vasopressin V2 receptor (V2R) or aquaporin-2 (AQP2). Identification of the genes involved and analysis of the cellular fate of the V2R and AQP2 mutants are relevant for understanding the functioning of the V2R and AQP2 protein. These developments also have implications for future therapeutic options.

The development of nonpeptide vasopressin receptor antagonists (VRAs) offers prospects for the treatment of euvolaemic (SIADH) or hypervolaemic hyponatraemia (congestive heart failure or cirrhosis). Several nonpeptide VRAs are now in various stages of clinical trials. At present, only conivaptan is registered by the FDA for intravenous treatment of euvolaemic and hypervolaemic hyponatremia. A recent long-term study comparing tolvaptan with placebo in patients with chronic heart failure showed no reduction in risk of death and hospitalisation.

KEYWORDS

Conivaptan, diabetes insipidus, SIADH, tolvaptan, vasopressin, water homeostasis

INTRODUCTION

Water is essential for living organisms. Human water balance is tightly regulated by the integrated action of osmoreceptors and volume receptors, thirst, vasopressin, and the excretion of water by the kidney.[1] Disturbances in water metabolism are frequent in clinical practice and include well-known syndromes such as diabetes insipidus and the syndrome of inappropriate secretion of antidiuretic hormone (SIADH). In recent years the pathophysiology of these water metabolism disorders has been largely unravelled, which has increased the prospects for new treatment modalities.

In this review we will discuss normal water regulation and specifically address recent advances in the understanding of the role of mutations in the vasopressin receptor and their relevance for future treatment strategies.

CURRENT CONCEPTS IN WATER REGULATION

The maintenance of human water balance depends on the regulation of water intake (which is governed by the sensation of thirst and the availability of water) and of water excretion in the kidney, which is under the control of the antidiuretic hormone arginine vasopressin (AVP), and dependent on the presence of a hypertonic medulla and functioning vasopressin type-2 receptors (V2R) and aquaporin-2 (AQP2) water channels.

In normal physiology, arginine vasopressin (AVP) is synthesised in the hypothalamus and is secreted into the circulation by the posterior pituitary gland, in response to an increase in serum osmolality or a decrease in effective circulating volume. In general, the plasma sodium concentration is the primary osmotic determinant of AVP release. The osmoreceptors (specialised hypothalamic cells) are extremely sensitive and respond to alterations in the plasma osmolality of as little as 1%.[2] The osmotic threshold for AVP is about 280 mosmol/kg. This system is so efficient that plasma osmolality does not vary by more

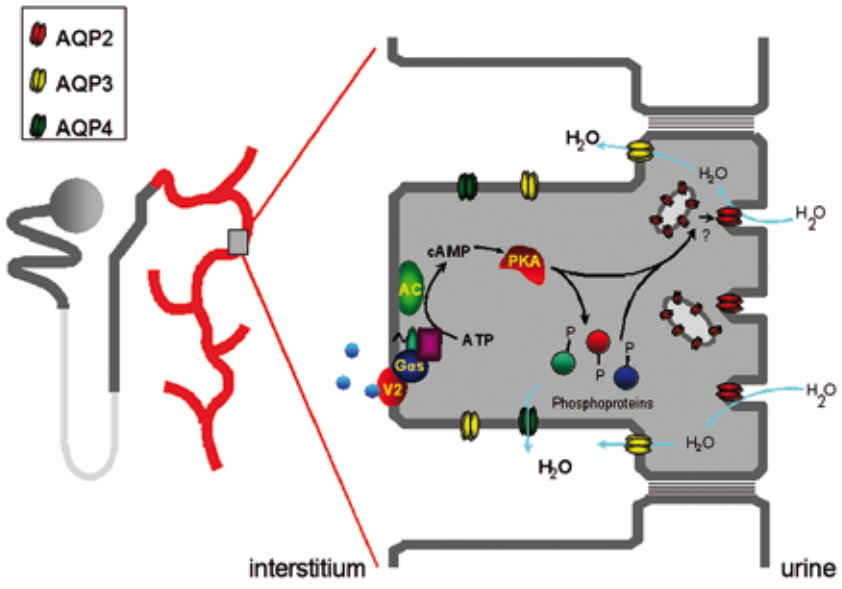
than 1 to 2%, despite wide fluctuations in water intake.[3] Although relatively stable in a healthy person, the set point of the osmoregulatory system may be lowered by pregnancy, the menstrual cycle, and relatively large, acute reductions in blood pressure or circulating volume.

The sensitivity of the volume receptors is different to that of the osmoreceptors. AVP is only secreted nonosmotically in humans if there is a large enough change in the effective arterial blood volume (approximately 5-10%), usually leading to a reduction in (standing) blood pressure.[4] In case of such large, significant blood volume changes, signals originating from the volume receptors override those originating from the osmoreceptors.[3] AVP secretion may also be stimulated by nausea, acute hypoglycaemia, glucocorticoid deficiency and smoking.[5] The primary stimulus for water ingestion is thirst, which is mediated by an increase in plasma osmolality (threshold 295 mosmol/kg) or a decrease in blood pressure or extracellular volume. Various hormones such as AVP and angiotensin II are involved in the stimulation of thirst.

AVP is rapidly metabolised in the liver and excreted by the kidney, with a half-life in the circulation of only 15 to 20 minutes. There are two major receptors for AVP: the V1 and the V2 receptors.

V1a receptors are located in vascular smooth muscle cells, cardiomyocytes, platelets, hepatocytes and myometrium.[6] The effect of activation of V1a receptors depends on their location. V1a receptor activation mediates vasoconstriction, vascular smooth muscle cell proliferation, platelet aggregation, glycogenolysis and uterine contraction in accordance with the above-mentioned locations. The V1a receptor also promotes hypertrophic growth of myocardial cells. Antagonism may result in increased cardiac output, reduced total peripheral resistance and reduced mean arterial blood pressure. V1b receptors are localised in the anterior pituitary gland and facilitate the release of ACTH. V2 receptors are located in the vascular endothelium and in the basolateral membranes of the principal cells in the cortical and medullary collecting tubules. Activation of the endothelial V2 receptor increases the release of factor VIII and von Willebrand factor, an effect which is used to attenuate an increased bleeding tendency by administering the V2 receptor agonist dDAVP. Binding of AVP to the V2 receptor in the kidney activates a G-protein mediated signalling pathway, involving activation of adenylate cyclase and the formation of cyclic adenosine monophosphate (cAMP). The subsequent activation of protein kinase A promotes the translocation of AQP2 water channels from cytoplasmic vesicles to the apical membrane (*figure 1*). Consequently, this usually watertight membrane becomes water permeable, thereby allowing the transcellular passage of water along the favourable osmotic gradient, and through the aquaporin-3 and aquaporin-4 water channels, which are present in the basolateral membrane. In response to decreased blood AVP levels, the AQP2 water channels are modified by ubiquitination, a process in which ubiquitin peptides are attached to AQP2 proteins.

Figure 1. *Regulation of renal water homeostasis by vasopressin (original figure from reference 15, reprinted with permission of the American Physiological Society)*



A nephron with a magnified principal cell is shown. Binding of AVP to the V2 receptor in the kidney activates a G-protein mediated signalling pathway, involving activation of adenylate cyclase and the formation of cyclic adenosine monophosphate (cAMP). The subsequent activation of protein kinase A promotes the translocation of AQP2 water channels from cytoplasmic vesicles to the apical membrane in the cell. The membrane becomes water permeable, thereby allowing transcellular passage of water along the favourable osmotic gradient and through AQP3 and AQP4 water channels (which are present in the basolateral membrane). For further details, see text.

Ubiquitin is a small protein designed to mark other proteins for intracellular destruction. Destruction, or more precisely proteolysis, occurs in a barrel-shaped structure, the proteasome. Ubiquitin can also mark transmembrane proteins, such as receptors, for removal from membranes. Thus, AQP2 water channels are removed from the luminal membrane by endocytosis and returned to cytoplasmic vesicles.[7] Apart from this direct effect on water transport, AVP also increases sodium transport via the epithelial sodium channel (ENaC) and urea transport through the UT-A1 transporter.[8,9]

DISORDERS OF WATER METABOLISM

Disturbances in water homeostasis can be divided into disorders of urinary concentration and disorders of urinary dilution (*table 1*). The major examples of such disorders are SIADH and diabetes insipidus.

SIADH is characterised by a nonphysiological release of AVP. It is a common problem that can be seen in a wide variety of clinical situations. SIADH manifests itself as an inability to excrete a free water load, with inappropriately concentrated urine, resulting in hyponatraemia and hypo-osmolality. The hyponatraemia is initially mediated by AVP-induced water retention, the ensuing volume expansion results in sodium losses. SIADH may result from enhanced hypothalamic secretion of AVP, ectopic production of AVP (malignancies), or the administration of exogenous AVP or oxytocin. Certain forms of drug-induced SIADH may be due to an increased susceptibility to AVP.[10] Recently a genetic form of SIADH was described, which could be attributed to a gain-of-function mutation of the vasopressin 2 receptor due to a point mutation in the V2R gene.[11] The result of this mutation is an AVP-independent continuous activation of the V2 receptor, which increases urinary concentration. This condition has been referred to as nephrogenic syndrome of inappropriate antidiuresis (NSIAD). Osmotically inappropriate antidiuresis may also be caused by stimuli such as hypovolaemia, hypotension (cirrhosis, congestive heart failure) or glucocorticoid deficiency. In patients with congestive heart failure (CHF) or cirrhosis, water retention is an initially beneficial defence against decreased cardiac output in CHF or the dilated splanchnic circulation in cirrhosis. Current and future treatment modalities in SIADH are presented in *table 1*. The very recent development and use of AVP type 2 receptor antagonists will extend our therapeutic armamentarium (see below).

Diabetes insipidus (DI) is characterised by the production of abnormally large volumes of urine that result from either a decreased secretion of AVP (central DI) or a diminished responsiveness to AVP (renal or nephrogenic DI). Patients complain of thirst and polyuria, but clinical signs of dehydration are uncommon unless fluid intake is impaired or prohibited. Central DI can be caused by a variety of congenital, acquired or genetic disorders, though in nearly 50% of cases it is idiopathic (*table 1*). Nephrogenic DI (NDI) is a congenital or acquired disorder in which hypothalamic function and AVP release are normal, but the ability to concentrate urine is reduced because of diminished or absent renal responsiveness to AVP. Acquired NDI is most often caused by electrolyte disturbances (hypocalcemia or hypokalaemia), urinary tract obstruction or exposure to certain drugs (e.g. lithium).

The genetic forms of NDI are related to mutations in the AVP V2 receptor or AQP2 gene.[12-15] The gene for the AVP V2 receptor is located on the X-chromosome, thus NDI caused by mutations in the V2 receptor gene are inherited and transmitted in an X-linked mode. The AQP2 gene is located on chromosome 12,[16] and NDI caused by mutations in the AQP2 gene is inherited in an autosomal mode. Both autosomal dominant and recessive forms have been described. Recent studies have provided more insight into the impact that various mutations have on the functioning of the V2 receptor and the AQP2 protein. Insight into these mechanisms provides clues for future therapeutic interventions, as is evident from the studies concerning the V2 receptor that will be addressed in the next section.

NEPHROGENIC DIABETES INSIPIDUS AND MUTATIONS IN THE V2 RECEPTOR

Likely due to its location on the X-chromosome, 90% of the patients suffering from congenital NDI have mutations in the V2R gene. Currently, more than 180 V2R gene mutations have been described,[17] and all these mutations result in severely disturbed receptor signalling that corresponds to an insensitivity of the renal principal cell for AVP. The molecular mechanism causing this insensitivity differs among mutants and recent data indicate that one of these underlying mechanisms might be treatable in the future.

The V2R belongs to the large family of G-protein coupled receptors (GPCR) and, based on the underlying mechanism, GPCR gene mutations in general and V2R gene mutations in particular were recently divided into five different classes according to their cellular fate (*figure 2*).[18,19]

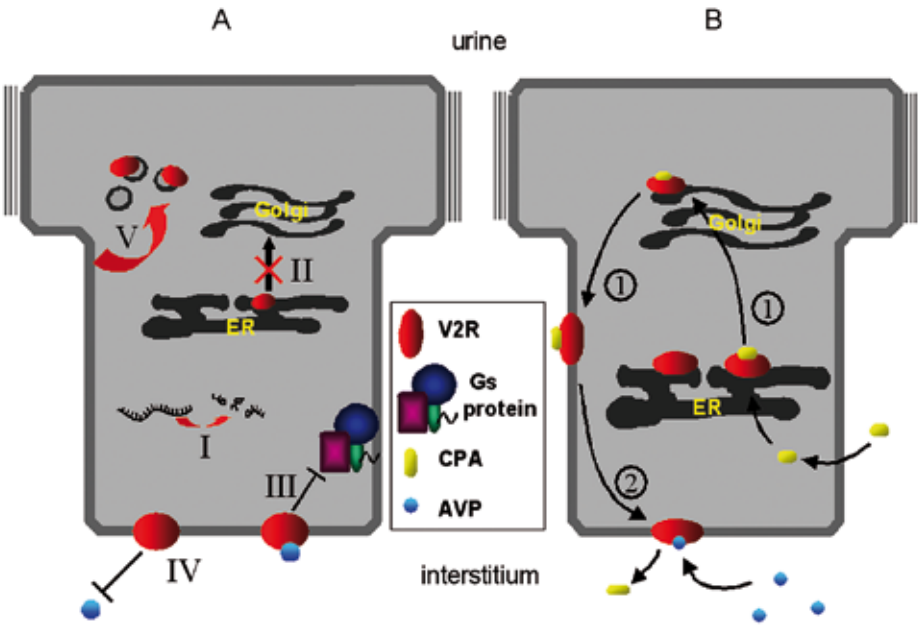
Class I comprises mutations that lead to improperly processed or unstable RNA, resulting in the absence of a fully translated protein. Class II mutations are mainly missense mutations resulting in fully translated proteins, but due to a point mutation the receptors are misfolded. Instead of being transported to the plasma membrane, they are retained in the endoplasmic reticulum (ER). This

Table 1. *Overview of disorders in water metabolism*

Primary defect	**Reduced urinary concentration**				**Reduced urinary dilution**			
Disorder	Central diabetes insipidus (CDI)	Nephrogenic diabetes insipidus (NDI)			Syndrome of inappropriate ADH secretion (SIADH)	Volume-dependent ADH secretion (SIADH-like)	Nephrogenic syndrome of inappropriate antidiuresis (NSIAD)	
Pathogenesis	Complete or partial failure of vasopressin secretion	Reduced renal responsiveness to AVP due to defective V2 receptor	Reduced renal responsiveness to AVP due to defective AQP2	Reduced renal responsiveness to AVP due to absence of medullary gradient	Enhanced secretion of vasopressin or potentiation of vasopressin effect	Enhanced secretion of vasopressin due to a decreased effective volume	Increased renal responsiveness to AVP due to a gain of function mutation of the V2 receptor	Impaired dilution due to decreased NaCl re absorption in the distal tubule
Causes	*Acquired* Idiopathic Neurosurgery Head trauma Neoplastic Hypoxic or ischaemic encephalopathy Other (sarcoidosis, histiocytosis X etc.) *Congenital* CNS malformations	*Congenital* X-linked loss of function mutation in V2 receptor gene	*Congenital* Autosomal recessive or autosomal dominant mutations in aquaporin 2 gene *Acquired* Lithium Hypercalcaemia Hypokalaemia	*Congenital* Defective Na-K-Cl cotransporter in thick ascending limb (Bartter) *Acquired* Sickle cell disease Urinary tract obstruction Loop diuretics Cisplatinum Sarcoidosis	Increased hypothalamic production (neuropsychiatric disorders, drugs, pulmonary disease, postoperative etc.) Ectopic production (carcinoma) Exogenous ADH Carbamazepine Chlorpropamide Hypothyroidism* Adrenal insufficiency*	Cirrhosis Congestive heart failure	Missense mutation of V2 receptor (R137C, R137L)	*Congenital* Defective Na-Cl cotransporter due to autosomal recessive mutation in SLC12A3 gene (M. Gitelman) *Acquired* Inhibition of NaCl reabsorption due to thiazide diuretic
Treatment	dDAVP Thiazide diuretic and/or amiloride Chlorpropamide Carbamazepine Clofibrate	Future therapies **V2 receptor-antagonists acting as chaperones** Activation of cGMP kinase	*Congenital* Thiazide diuretic or amiloride Low sodium and protein diet NSAID (dDAVP) *Acquired* Resolve underlying disorder and see above	*Congenital* NSAID *Acquired* Treat underlying disorder if possible or stop causative agent	Water restriction Intravenous administration of hypertonic saline NaCl tablets Loop diuretics High salt, high protein diet Demeclocycline Lithium Urea Vasopressin receptor antagonists	**V2 receptor antagonists** Loop diuretics Water restriction	Water restriction Demeclocycline Lithium Urea V2 receptor antagonist acting as inverse agonists (only tested *in vitro*)	Water restriction Withdrawal of thiazide diuretic

*Hypothyroidism and adrenal insufficiency cause SIADH-like syndrome, the exact mechanisms involved are unresolved but likely to include inappropriate ADH secretion or enhanced sensitivity to ADH.

Figure 2. *Cellular fate of V2R mutants in congenital nephrogenic diabetes insipidus (NDI) and their rescue (original figure from reference 15, reprinted with permission of the American Physiological Society)*



A) Classes of V2R mutations in NDI. In the schematic view of the collecting duct cell, the fate of the different mutation classes is indicated by Roman numerals. Mutations can lead to incomplete proteins (I); endoplasmic reticulum (ER) retention of full-length proteins (II); impaired Gs coupling (III); impaired AVP binding (IV); and missorting in the cell (V). See text for details.
B) Rescue of class II V2R mutants by pharmacological chaperones. Class II V2R mutants are retained in the ER, but on binding of cell-permeable antagonists (CPA), the wild-type structure of the V2R mutant is stabilised, which allows them to exit the ER, mature in the Golgi complex, and subsequently translocate to the plasma membrane (1). At the membrane, displacement of the CPA by AVP will allow activation of the cAMP cascade (2), the missing step needed for translocation of AQP2 to the apical membrane.

organelle not only synthesises membrane proteins, but also assures that these proteins are properly folded and assembled. Misfolded proteins, including V2R mutants, are subsequently degraded by the proteasome.[20]

Class III and IV mutations also result in fully translated proteins. These proteins bypass the quality control performed in the ER and are transported to the plasma membrane. In the plasma membrane class III mutants disturb the binding of the stimulatory Gs protein, leading to reduced activation of adenylate cyclase and formation of cAMP, while class IV mutants are unable to bind AVP. Finally, class V mutations allow normal protein synthesis and maturation, but here the protein mutations direct the mutant receptor to another organelle in the cell. Due to mislocation, the V2R mutant is only briefly available for AVP binding.

Intracellular retention of V2R missense mutants in the ER and their rapid degradation likely represent the main cause for NDI. The extent of ER retention may differ among mutants and may represent differences in their folding state. This was illustrated by Hermosilla *et al.*, who found that only three of eight V2R mutants in NDI were strictly kept in the ER, whereas the other five were transported to the ER-Golgi intermediate compartment, followed by retrograde transport to the ER.[21]

Although several mutants belong to one class only, others display characteristics of several classes. Some, for example, are partially ER retained (class II), whereas another fraction is expressed at the plasma membrane and exerts a reduced activity (class III or IV).[19] This partial expression of these mutants at the plasma membrane may explain the observed small antidiuretic response to high doses of desmopressin in NDI patients encoding these mutants.[23]

PHARMACOLOGICAL CHAPERONES TO RESCUE CLASS II V2R MUTANT

While cell biological studies have revealed that most V2R mutants in NDI are retained in the ER, other studies have proved that many of these ER-retained receptors are intrinsically functional (i.e. are able to bind AVP and generate a cAMP cascade). This means that ER retention is the main reason for these mutant receptors to cause NDI and that drugs that can rescue their cell surface expression may be of therapeutic value.[24-27] As such, the discovery that cell-permeable V1R and V2R antagonists are able to enter the cell, stabilise the V2R mutant and rescue its cell surface expression (*figure 2*) was widely hailed as a breakthrough. Most though not all ER-retained V2R

mutants are rescued and re-expressed at the cell surface by these 'pharmacological chaperones'. To be of therapeutic use, the rescuing antagonist needs to be displaced by AVP after translocation of the V2R mutant to the basolateral membrane. Since the competition between AVP and the rescuing ligand at the plasma membrane determines functional rescue, low-affinity ligands are deemed to be more successful than high-affinity antagonists.[28] Indeed, Bernier *et al*. recently found that administration of a low-affinity V1R antagonist resulted in a small but significant reduction in urine production and water intake in five NDI patients with class II mutations, thereby proving in principle that pharmacological chaperones can also rescue V2R mutant activity *in vivo*.[29] Of great importance is that this treatment with relatively high blood concentrations of V1R antagonist came with a minimum of side effects. *In vitro* data show that at clinically relevant low concentrations of antagonists, V2R antagonists yield a better functional rescue of V2R mutants than V1R antagonists.[27] It is therefore anticipated that these drugs reduce NDI to a greater extent than V1R antagonists. As several of the V1R and V2R antagonists have been or are close to FDA approval, it will be exciting to discover to what extent they are able to relieve NDI caused by class II functional V2R mutants.

AVP RECEPTOR ANTAGONISTS

Conventional therapies for euvolaemic (SIADH) or hypervolaemic hyponatraemia (congestive heart failure (CHF), cirrhosis) including water restriction, hypertonic saline, democycline and urea are moderately effective and their use is limited by noncompliance or side effects. None of these therapies directly addresses the pathogenetic mechanism involved, i.e. elevated plasma AVP. Many patients with CHF develop hyponatraemia and hypervolaemia that is poorly responsive to conventional loop diuretics. Hyponatraemia in CHF is associated with increased morbidity and mortality, underlining the importance of adequate correction of this electrolyte disorder. The development of nonpeptide vasopressin receptor antagonists (VRAs) has opened a new era in the treatment of hyponatraemia. Several of these nonpeptide VRAs are now in various stages of clinical trials.

Tolvaptan is a selective V2 receptor antagonist. Schrier *et al*. reported the combined data of two randomised trials evaluating 225 outpatients with euvolaemic or hypervolaemic hyponatraemia, who were given 15 and later 30 or 60 mg tolvaptan over a period of 30 days.[30] Tolvaptan increased average serum sodium concentration by 4 to 5 mmol/l throughout the entire treatment period. About 25% of the patients withdrew from the trial due to side effects. Similar results were obtained with conivaptan, which is the only combined V1a and V2 receptor antagonist that has been approved by the FDA for treatment of euvolaemic hyponatraemia. A randomised placebo-controlled trial in patients with euvolaemic or hypervolaemic hyponatraemia (n=74) showed that serum sodium normalised or increased >6 mmol/l within five days in 48% of patients who received placebo treatment, in 71% of patients who received 40 mg conivaptan, and in 82% of patients who were given 80 mg conivaptan.[31] In this short-term study, 5% withdrew from the trial because of side effects. Both studies noted hypotension, nausea, thirst, constipation, and dry mouth as the main side effects. Two other selective V2 antagonists, lixivaptan and SR121463, are currently being investigated in phase III trials.

From a clinical perspective, VRAs might become important new drugs for the treatment of patients with congestive heart failure. In patients with heart failure AVP is stimulated, thus causing hyponatraemia and impairment of diuresis. Two major randomised trials studying the effect of tolvaptan in CHF have been carried out. A randomised controlled trial studied 254 patients with CHF (NYHA II-III) and compared the effect of three different oral doses of tolvaptan for a duration of 25 days.[32] Tolvaptan-treated patients showed a decrease in body weight and had small, but significant increases in serum sodium concentrations. This effect gradually decreased over 25 days. Another major trial randomised 319 patients, who required hospitalisation for CHF, to receive placebo, 30, 60 or 90 mg tolvaptan for 60 days.[33] The primary endpoint was weight loss after 24 hours, which was greater in the tolvaptan group. Additional primary endpoints were worsening CHF, death or re-hospitalisation. Although there were no differences among the treatment groups, in post hoc analysis, 60-day mortality was significantly lower in the tolvaptan-treated patients with renal dysfunction or severe congestion at baseline. Considering these data, long-term benefits of VRAs in patients with CHF could be expected. The recent outcome of the EVEREST trial has therefore been somewhat disappointing.[34] The EVEREST trial tested the benefit of tolvaptan, given once a day (30 mg *vs* placebo) in two identical short-term studies and a longer-term safety and outcome trial. A total of 4133 patients were randomised. Although the short-term studies showed a change in body weight in favour of tolvaptan and modest improvement in dyspnoea and oedema, the long-term trial (treatment for a minimum of 60 days, median follow-up 9.9 months) demonstrated no reduction in risk of death or hospitalisation. A total of 537 patients in the tolvaptan group (25.9%) and 543 patients in the placebo group (26.3%) died (p=0.68). The other primary endpoint of hospitalisation or death from cardiovascular causes occurred in 871 patients in the tolvaptan group (42.0%) and 829 patients in the placebo group (40.2%, p=0.55). No significant worsening of renal function was observed; the

main side effects of tolvaptan were dry mouth and thirst. Although tolvaptan can be safely used in patients with CHF, the lack of an effect on primary endpoints and the side effects advocate against its long-term use.

Theory and empirical evidence obtained from animal experiments indicate that dual vasopressin V1a and V2 receptor antagonists might provide greater benefits in patients with CHF than selective V2 receptor antagonists.[35] V1a receptor antagonists attenuate vascular smooth muscle contraction, resulting in vasodilatation and a decrease of systemic vascular resistance. As a consequence, the cardiac output may increase. Indeed in an acute study intravenously administered conivaptan lowered pulmonary capillary wedge pressure and right atrial pressure and increased urinary output in patients with CHF.[36] No animal or human experiments studying the haemodynamic effects of tolvaptan have been conducted so far, nor have there been trials comparing conivaptan with tolvaptan. Currently a large multicentre trial with hard endpoints is underway to examine the long-term benefits of conivaptan on exercise tolerance in patients with CHF.[37] It should be noted that conivaptan has only been approved for intravenous use by the FDA. Until data from additional clinical trials become available, VRAs are not recommended in patients with CHF.

CONCLUSION

In the past years our understanding of AVP-mediated urinary concentration has substantially improved with the elucidation of the crucial molecular mechanisms involved. Research on congenital NDI has led to promising therapeutic strategies that mediate an antidiuretic response. Advantageous effects of vasopressin antagonists have resulted in FDA approval of conivaptan for intravenous treatment of patients with euvolaemic or hypervolaemic hyponatraemia. However, in patients with congestive heart failure the expected beneficial effect of long-term use of a selective V2 receptor antagonist on risk of death or hospitalisation was not observed.

ACKNOWLEDGMENT

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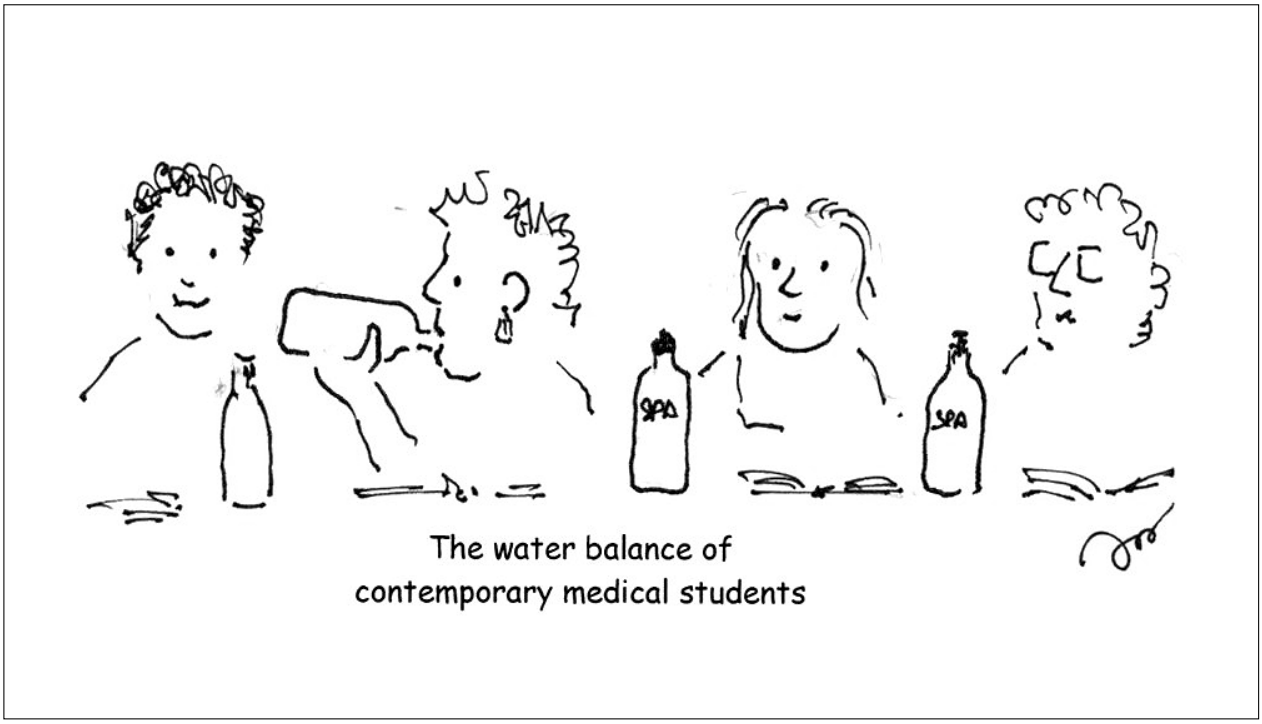

Peters, et al. New aspects of disturbances in water metabolism.

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ORIGINAL ARTICLE

Experience with alemtuzumab in treatment of chronic lymphocytic leukaemia in the Netherlands

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ABSTRACT

Background: Alemtuzumab (MabCampath®) is a monoclonal antibody against CD52, indicated as third-line treatment of chronic lymphocytic leukaemia (CLL). As most important side effect opportunistic infections are mentioned. It is, however, unknown whether these complications often lead to problems in general patient care in the Netherlands.

Methods: To gain insight into the use and complications of alemtuzumab therapy, the alemtuzumab-treated CLL patients in 15 hospitals in the Netherlands were evaluated by means of a questionnaire.

Results: In the period from 31 October 2001 until 17 November 2005, 27 patients with CLL or prolymphocytic leukaemia (PLL), RAI stage I to IV, Binet stage A to C, received 32 treatments with alemtuzumab. The time from diagnosis until start of alemtuzumab treatment was 6 ± 4.5 years (mean ± SD). The treatment lasted 11 ± 7 weeks. Of the treatments, 41% could be administered for the full 12 weeks.

The most frequent adverse events were fever (72%), shivering (47%), fatigue (22%) and dyspnoea (16%). Haematological side effects consisted of leucopenia (75%), thrombocytopenia (44%), and anaemia (13%). Infectious complications occurred in 12 of 32 (38%) treatments: pneumonia (25%; of which one *Pneumocystis carini* pneumonia and four *Aspergillus* infections), sepsis (9%; of which one *Listeria*), herpes zoster (9%), herpes simplex (6%), CMV reactivation (6%), meningitis (3%) and Guillain Barre (3%).

The overall response was 53%, with complete remission in 13%, partial remission in 41%, stable disease in 25% and progressive disease in 13%, and lasted for 8.3 ± 7.3 months.

Conclusion: Treatment with alemtuzumab is often terminated prematurely, leading to a suboptimal treatment effect. Fear of severe uncontrollable opportunistic infections seems unjustified.

KEYWORDS

Alemtuzumab, CLL, infections, side effects

INTRODUCTION

Chronic lymphocytic leukaemia (CLL), with an incidence of 3 to 5 per 100,000 per year, is the most common form of leukaemia in adults. Survival depends on the clinical stage, the presence or absence of somatic mutations in genes coding for the heavy chain of immunoglobulins (IgV_H genes)[1] and cytogenetic abnormalities.[2]

At present the first line of treatment for CLL is chlorambucil. When progression occurs fludarabine or combination chemotherapy is chosen as the next line of therapy. Patients who have become resistant to fludarabine have a higher risk of infection and an unfavourable prognosis, with a median survival of only ten months.[3] Recent developments with monoclonal antibodies open new perspectives for third-line treatment of this unfavourable prognostic group of CLL patients.

Alemtuzumab (Mabcampath®) is a monoclonal antibody targeted to CD52, an antigen present on both normal and malignant B and T lymphocytes and on monocytes, thymocytes and macrophages. Binding of alemtuzumab to CD52 initiates complement activation via the classical pathway. The membrane attack complex which is formed in this way leads to lysis of the lymphocyte. Besides this complement-dependent cytotoxicity (CDC), alemtuzumab also works by an antibody-dependent cellular cytotoxicity (ADCC), by forming a complex between CD52-positive cells and Fc receptors on NK cells, monocytes and macrophages, leading to cell destruction. As a third mechanism of action alemtuzumab induces apoptosis of CD52-positive cells.[4,5]

At present alemtuzumab is not only indicated as third-line treatment of CLL after failure of conventional treatment including fludarabine, it is now also being used upfront in the first-line in a randomised Dutch study of high-risk CLL patients. The response rate reported in the literature is 33%; 2% complete remission (CR) and 31% partial remission (PR).[6] Acute, infusion-related side effects are fever, rigors/chills, nausea, vomiting, hypotension, rash, dyspnoea, cough and diarrhoea. Haematological toxicity with pancytopenia and infections are more long-lasting complications. Opportunistic infections, such as *Pneumocystes carinii* pneumonia and cytomegalovirus (CMV) pneumonitis, have been reported as the most important side effect of alemtuzumab.

It is not clear whether these complications often lead to problems in clinical care. To gain insight into the use and complications of alemtuzumab therapy in the Netherlands, the treatment of fludarabine-resistant CLL patients with alemtuzumab was evaluated using a questionnaire.

MATERIALS AND METHODS

With the help of Schering BV, a list of physicians and accompanying hospitals in the Netherlands that had prescribed alemtuzumab in the period from 2001 until 2005 was composed. These physicians were approached for a retrospective investigation of the medical records of CLL patients treated with alemtuzumab in the above-mentioned period. The investigation was performed by means of a questionnaire, constructed on the basis of the literature.[6]

Information was collected on the demographical characteristics of the patients, the clinical stage of CLL at the start of treatment, cytogenetic abnormalities and IgV_H mutational status. Previous treatments including fludarabine treatment and whether or not patients were fludarabine resistant, which was defined as no response to or progression during or within six months after fludarabine, was recorded. The duration and intensity of the treatment with alemtuzumab was also described.

The treatment effect of alemtuzumab was reported as complete remission (CR), partial response (PR), stable disease (SD) or progressive disease (PD) according to the National Cancer Institute-Sponsored Working Group Guidelines for Chronic Lymphocytic Leukaemia.[7]

Side effects were reported according to the common toxicity criteria.[8] (Sub)acute infusion-related side effects, haematological toxicity and infectious complications were recorded. Infections were defined as the state produced by the establishment of an infective agent in or on a suitable host as assessed by the responsible physician and it was attempted to specify *Aspergillus* infections in possible, probable and proven ones.[9] *Pneumocystes carinii* pneumonia was defined as an opportunistic infection possibly caused by *P. carinii* characterised by a nonproductive cough, shortness of breath, fever, bilateral interstitial infiltrates and hypoxaemia and responding to treatment aimed at this infection.

RESULTS

Thirteen of the 28 hospitals approached for this investigation dropped out. Nine hospitals had not treated CLL patients with alemtuzumab, in one hospital the treatment of the only patient had just started, and three hospitals refused to cooperate. The remaining 15 hospitals, one university hospital, six teaching hospitals and eight general hospitals reported on all their consecutive CLL patients treated with alemtuzumab, a total of 29 patients.

Two of these 29 patients were not included, one because he had not been treated with alemtuzumab and one because no information on the treatment with alemtuzumab could be recovered. The other 27 patients, mean age 63 years (range 49-77), 20 male, 7 female, received 32 treatments with alemtuzumab from 31 October 2001 until 17 November 2005. In these patients the diagnosis was made of either CLL (24; 89%) or PLL (3; 11%), Rai stage I (2; 7%), stage II (2; 7%), stage III (5; 19%), stage IV (17; 63%), unknown (1; 4%), Binet stage A (1; 4%), stage B (3; 11%), stage C (22; 82%), and unknown (1; 4%). Cytogenetic abnormalities were sparsely recorded. Cytogenetics were normal in four patients, one had a 6 q deletion, one a 13q deletion, and one patient had a 13q deletion, an 11q deletion and a 17p deletion. In the other 20 patients cytogenetics were not performed. The IgV_H mutational status was not known in any of the patients.

On average, patients had received three lines of previous treatment (range 0-8). Twenty-three patients (85%) had received fludarabine previously, 16 (59%) chlorambucil, 15 (56%) cyclophosphamide, vincristine and prednisone (CVP) and 12 (44%) patients had received chlorambucil combined with prednisone before. One patient (4%) had not received prior therapy and received alemtuzumab as upfront treatment. Twenty patients (87% of the fludarabine-treated patients) were fludarabine resistant.

The time from diagnosis until start of alemtuzumab treatment was 6 ± 4.5 years. In 27 of 32 treatments (84%) the loading dose of 3, 10, 30 mg was given, in one patient 3 mg was administered twice and for four treatments the loading dose could not be retrieved. Of the treatments, 24 (75%) followed the recommended dosage of 30 mg three times weekly for four to 12 weeks. In three of the treatments (9%) the highest achievable dosage was less than 30 mg, namely 10 mg. In five treatments (16%) the highest achievable dosage was unknown. All other 24 (75%) treatments reached the intended dosage of 30 mg. In two treatments the dosage had to be reduced to 10 mg, once because of

thrombocytopenia and once because of thrombocytopenia and anaemia; this dose reduction was effective as both the thrombocytopenia and anaemia recovered. In 28 (88%) treatments the frequency of administration was three times weekly, for the remaining four treatments the frequency of administration was unknown.

Median follow-up was 13 months (range 2-37). Treatment lasted 11 ± 7 weeks, with a minimum of two and a maximum of 42 weeks; this last treatment was given together with fludarabine once every three weeks. The therapy was terminated prematurely in 17 treatments (53%); prematurely was defined as shorter than 12 weeks. The reason for early termination of treatment could not be retrieved in three cases (18 %). In five treatments (29%) the treatment was stopped because of fever or other side effects, in three (18%) there was progressive disease, in two (12%) complete response, in two (12%) severe haematological toxicity, in one (6%) haemolytic anaemia and one patient went on for allogeneic bone marrow transplantation. The treatment could be completed in 13 cases (41%) (for 12 weeks or longer), from two patients (6%) the duration of treatment could not be recovered. Alemtuzumab was predominantly (18 treatments, 56%) administered intravenously, in three treatments (9%) subcutaneous administration was used and in 11 treatments (34%) the route of administration was unknown.

Efficacy of alemtuzumab

Best response to alemtuzumab is described in *table 1*. The overall response rate was 53%. The duration of the response was 8.3 ± 7.3 months. One patient (4%) died while on treatment with alemtuzumab. The cause of death is unknown. Six patients died within six months after the start of alemtuzumab treatment, the cause of death was not retrievable in five patients, in one patient it was due to progressive disease and an *Aspergillus* infection. In total 13 of 27 (48%) patients have died. Of seven patients the cause of death could not be recovered, in one patient it was due to progressive disease, one patient died of an *Aspergillus* infection, in three patients death was due to both progressive disease and an infection (one *Aspergillus* infection, one *Listeria* infection and one meningitis with unknown pathogen) and one patient died of graft-versus-host-disease after allogeneic bone marrow transplantation.

Table 1. *Efficacy of alemtuzumab*

Response	Treatments (n=32)	% of total number of treatments
Complete remission	4	13
Partial response	13	41
Stable disease	8	25
Progressive disease	4	13
Unknown	3	9

Patients died on average nine months after termination of alemtuzumab treatment (minimum 2 weeks, maximum 20 months).

(Sub)acute side effects to alemtuzumab

Side effects occurring during or directly after administration of alemtuzumab are described in *table 2*. Fever was the most frequent. In seven of 32 treatments fever recurred with every administration of alemtuzumab, in two of 32 it only occurred in the first three weeks of treatment and in three only in the first two weeks of treatment.

Haematological side effects to alemtuzumab

Leucopenia occurred in 24 of 32 (75%) treatments with alemtuzumab. Thrombocytopenia occurred or aggravated in 14 of 32 (44%). Two (6%) of these were clinically relevant, with the number of platelets <10 x 10^9/l. In one patient a thrombocytopenia grade IV was present before the start of treatment with alemtuzumab. The nadir of thrombocytes was on average encountered after four weeks

Table 2. *Toxicity of alemtuzumab*

Side effects	Occurring in no. of treatments (n=32) (%)	Grade 1	Grade 2	Grade 3	Grade 4	Grade unknown
Subacute side effects						
Fever	23 (72)	-	6	7	1	9
Rigor/chills	15 (47)	-	6	2	-	7
Fatigue	7 (22)	-	1	5	1	-
Dyspnoea	5 (16)	-	1	2	1	1
Diarrhoea	5 (16)	1	1	1	2	-
Nausea	3 (9)	-	3	-	-	-
Injection site reaction	3 (9)	-	1	2	-	-
Rash	2 (6)	-	-	1	-	1
Vomiting	2 (6)	1	1	-	-	-
Headache	2 (6)	-	2	-	-	-
Haematological toxicity						
Anaemia	32 (100)	9	10	5	1	7
Thrombo-cytopenia	27 (84)	-	6	11	3	7
Infectious complications						
Pneumonia	8 (25)					
Sepsis	3 (9)					
Herpes zoster	3 (9)					
Herpes simplex	2 (6)					
Sinusitis	2 (6)					
CMV reacti-vation	2 (6)					
Otitis media	1 (3)					
Guillain-Barré	1 (3)					
Meningitis	1 (3)					
Candidiasis	1 (3)					

and recovered in almost all cases, in four treatments (13%) an improvement of thrombocyte count compared with the start of treatment was even achieved.
Anaemia was also present at the start of treatment in 25 cases. Anaemia aggravated in four treatments (13%) (measured from baseline to nadir), with a mean nadir four weeks after the start of treatment. Two of these four treatments resulted in an improvement in the anaemia at the end of treatment. In 10 of 32 treatments (31%) an improvement of haemoglobin count was eventually reached.

Infectious complications with alemtuzumab treatment
Pneumocystis carinii pneumonia prophylaxis with cotrimoxazole and cytomegalovirus prophylaxis with valaciclovir was given in 25 of 32 treatments (78%), from the remaining seven it is unknown whether prophylaxis was administered.
Infectious complications with alemtuzumab treatment are described in *table 2*. In 12 of 32 treatments (38%) infections occurred. The most frequently encountered infection was a pneumonia which occurred in eight of 32 treatments. During these eight treatments, four patients had only one pneumonia, two patients had two, one patient had three and one patient even had four pneumonias. The pathogens responsible for the pneumonias were *Pneumocystis carinii* in one, a fungal infection in three of which two were possible and one was a proven *Aspergillus* infection, a combination of a bacterial and fungal infection (possible *Aspergillus*) in one, a bacterial infection in two and for eight pneumonias the pathogen was unknown.
Sepsis occurred in three patients and was caused by an *E. coli*, a streptococcus group A and a *Listeria* species.
Viral infections occurred in five (16%) of patients, three herpes simplex and two herpes zoster infections.

DISCUSSION

In this study the effect and complications of alemtuzumab therapy in the treatment of CLL patients in the Netherlands was evaluated. Although most hospitals that were approached participated in this investigation, the results need to be critically appraised as some of the information could not be retrieved retrospectively.
The patients in this study where heavily pretreated, mostly including fludarabine, and were in an advanced stage of the disease at the start of alemtuzumab treatment. In this unfavourable prognostic group of patients with a median survival of ten months.[3,6] an overall response (OR) was reached of 53%, with an average response duration of nearly 8.5 months. Keating *et al*. treated 93 CLL patients, previously treated with fludarabine, with alemtuzumab and obtained an OR of 33%, with a response duration of 9.5 months.[6] However, in that study all patients were fludarabine resistant, whereas in ours only 11 were. Others have shown comparable results in previously treated CLL patients, an OR varying between 33 and 57%[10-14] and a median response duration of 12 to 15.4 months.[10,11]
In our study only one patient (4%) died during treatment with alemtuzumab. In total 13 of 27 patients (48%) died. In four of these patients an infection, probably related to the use of alemtuzumab, played a role in the death. Also in other studies relatively few patients died while on treatment with alemtuzumab, between 0%[10] up to 9%.[13] Mortality is predominantly seen after completion of the treatment and can rise to 68%.[6]
Alemtuzumab treatment was terminated prematurely in 53% of cases, in 47% due to side effects. The (sub) acute side effects such as fever and rigors/chills usually diminish during treatment[6,10-12] and are well controlled by paracetamol and an antihistamine. Treatment was seldom stopped definitively because of these side effects.[11] As in our study, these side effects are less often seen and less severe after subcutaneous administration.[15-17]
Haematological toxicity consisted of leucopenia, anaemia and thrombocytopenia. Anaemia aggravated in four treatments (13%) but in 31% it eventually improved. Thrombocytopenia occurred or aggravated in 14 (44%) treatments and recovered in almost all cases. In four treatments (13%) an improvement in the thrombocyte count was seen compared with the start of the treatment, an effect which is also seen in literature.[11]
The most important complications of alemtuzumab therapy are infections. In 25% of treatments one or more pneumonias were observed. Also in other studies especially pulmonary infections are described.[6,10-12] Although in eight of 15 pneumonias no pathogen was retrieved, five opportunistic infections were seen in the other seven, four fungal infections and one *Pneumocystis carinii* pneumonia.
In our study only two CMV reactivations were observed. However, a CMV-PCR was only performed in seven treatments, in 25 it was either not done or unknown. This incomplete information admits no reliable conclusions about CMV reactivation. In literature CMV reactivation varies between 7%[6] and 66%.[18,19]

Opportunistic infections seen after alemtuzumab therapy are partly related to the disease itself.[20,21] Patients who are resistant or partially responsive to fludarabine appear to have the highest risk of infections[22] and retain a severe immunodeficiency for a long period of time.[23] The risk of infections varied between 23 and 79% for patients previously treated for CLL,[11,19,24-29] while this was only 8.7% in patients treated with alemtuzumab as first-line therapy.[16] Therefore, it seems that the incidence of infections rises with the number of lines of treatment

and with less responsiveness, and can not be directly related to alemtuzumab treatment alone. At this moment a phase III study has started within the HOVON (Dutch Haemato-Oncology Association) study group on the treatment of previously untreated high-risk CLL patients with fludarabine, cyclophosphamide, with or without alemtuzumab.[30] This randomised trial will give insight into both the response to this combination therapy and the additional toxicity of alemtuzumab.

The experience with alemtuzumab treatment of CLL patients in the Netherlands is promising. A good response rate is reached in an unfavourable prognostic group of patients. The most important side effects are opportunistic infections. Effective monitoring and pre-emptive treatment of CMV reactivation and prevention of *Pneumocystis carinii* pneumonia with cotrimoxazole and herpes infections with valaciclovir is of vital importance to prevent serious complications.

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ERRATA

NETH J MED 2007;65(6):219-21
PHOTO QUIZ
Neck swelling following a vigorous neck massage

The third author's name was misspelled and the affiliation was not included. The correct heading should be:
A. Ceylan[1], T. Akçam[2], E. Karatas[3], F. Çelenk[1*]
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NETH J MED 2007;65(7):235-47
REVIEW
Prevalence and clinical significance of organ-specific autoantibodies in type 1 diabetes mellitus
L.C.G. de Graaff, J.W.A. Smit, J.K. Radder

On pages 240 and 242 'EMA-negative' was mentioned when this should have been 'PCA-negative'.

Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Maintenance treatment with budesonide 6 mg versus 9 mg once daily in patients with Crohn's disease in remission

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ABSTRACT

Background: In previous trials, budesonide 6 mg/day was able to prolong the time to relapse in patients with quiescent Crohn's disease and budesonide 9 mg/day was effective in active disease with limited side effects. The aim of this study was to compare the effectiveness of budesonide 9 mg *vs* 6 mg once daily on the maintenance of remission and occurrence of adverse events.

Methods: Double-blind, randomised trial in patients with Crohn's disease in remission. Patients were randomised to receive 6 mg/day or 9 mg/day of budesonide (Budenofalk®) without concomitant treatment for Crohn's disease. Endpoints were the time to relapse and relapse rates after one year.

Results: Seventy-six patients were randomised to 6 mg/day and 81 patients to 9 mg/day. Survival analysis showed no differences in the time to relapse. One-year relapse rates were not significantly different (6 mg group 24%; 9 mg group 19%). Any adverse event was reported in 61 and 68% of patients in the 6 mg and 9 mg groups, respectively; none of the 12 serious adverse events were drug related.

Conclusion: The one-year relapse rates were low and not significantly different between the group of patients treated with budesonide 6 mg *vs* 9 mg/day. Also, time to relapse and the number of adverse events were similar in both treatment groups.

KEYWORDS

Budesonide, Crohn's disease, IBD, RCT

INTRODUCTION

Crohn's disease is a chronic inflammatory disorder of the digestive tract. Medical treatment focuses primarily on the mucosal inflammation and corticosteroids are highly effective for the induction of remission of active Crohn's disease.[1,2] However, in various studies, the rate of relapses is high after withdrawal of corticosteroids. Long-term use of systemically active corticosteroids is associated with a substantial number of side effects, such as acne, moon face, hirsutism, buffalo hump, impaired glucose tolerance, mood disturbances and osteoporosis.[3] Furthermore, low doses of systemically active corticosteroids were ineffective for the prevention of relapses in previous studies.[4] The topically active synthetic steroid budesonide may overcome these disadvantages of long-term treatment with corticosteroids in Crohn's disease. Budesonide combines high intrinsic corticosteroid receptor affinity with a strong first pass effect in the liver of about 90% after oral administration.[5,6] Two oral formulations have been developed to release budesonide in the ileum and proximal colon,[7] or in the ileum and majority of the colon.[8-10]

In mild and moderately active Crohn's disease within the ileum or ascending colon, budesonide capsules in a dose of 9 mg/day have proven efficacy.[11-14] In a Cochrane systematic review, the efficacy was almost comparable with prednisone regimens with significantly less corticosteroid-associated adverse events.[15] Furthermore, treatment with budesonide capsules is able to prolong the time to relapse in patients with Crohn's disease in clinical remission. In two out of three dose-finding studies, 6 mg/day was

superior to 3 mg/day and to placebo with respect to the time to relapse.[16-18] However, relapse rates in these groups treated with budesonide 3 or 6 mg/day were not significantly lower compared with placebo at the end of a one-year treatment period. These findings were confirmed by a pooled analysis of these three studies and a similarly designed trial with two parallel groups (placebo and 6 mg/day).[19,20] In the pooled analysis, the median time to relapse was significantly prolonged from 154 days in the placebo group to 268 days in the budesonide 6 mg/day group. The relapse rate after one year was 59% in the placebo group, which was not significantly different from 51% in the patients treated with budesonide 6 mg daily. Since a dose relationship seems to exist in these maintenance trials, administration of budesonide in a higher dose of 9 mg/day may be more effective. The primary objective of the study was to evaluate if the time to relapse is prolonged under budesonide 9 mg/day compared with 6 mg/day, in patients with Crohn's disease in remission at study entry. Secondary objectives were to evaluate the percentage of patients in remission and to examine the safety of budesonide 6 mg and 9 mg/day over a treatment period of one year.

MATERIALS AND METHODS

Patient selection

Patients aged between 18 and 75 years with confirmed Crohn's disease were eligible for the study if they fulfilled the following criteria: Crohn's disease in remission for at least three months, but not more than 18 months, remission was defined as a Crohn's Disease Activity Index (CDAI) below 150 points;[21] disease locations previously confined to the ileum or colon except rectal and perianal disease. Patients who fulfilled one of the following criteria were not eligible for the study: bowel surgery within six months before randomisation; history of small bowel resections exceeding 80 cm; disease locations proximal to the ileum; severe hepatic disease defined by elevated liver enzymes of three times the upper normal limit, or renal disease with serum creatinine levels more than twice the upper normal limit; presence of diseases that may deteriorate due to corticosteroids (such as diabetes mellitus, glaucoma, aseptic bone necrosis, acute psychosis and severe hypertension); need for parenteral nutrition; presence of active systemic infections or gastroenteritis; and pregnancy or inadequate use of contraceptives during the trial.

This study was conducted in accordance with the ICH Harmonised Tripartite Guideline for Good Clinical Practice (1996). Before recruitment of patients, the protocol was reviewed and approved by the local ethics committees. All patients gave their written informed consent before participation in the study.

Study drug and concomitant medication

Capsules containing pH-modified release pellets of budesonide (Budenofalk® 3 mg capsule) and placebo capsules with an identical appearance were manufactured by Losan Pharma GmbH, Neuenburg, Germany and supplied by Dr. Falk Pharma GmbH, Freiburg/, Germany. Patients were assigned to one of the two treatment groups by a randomisation list generated by the Rancode+ programme (version 3.6) of IDV, Gauting (Germany). Randomisation tables were stored in closed, nontransparent envelopes to be opened after closure of the database. For emergency reasons, closed envelopes containing the type of treatment were available on the site. These emergency envelopes were collected at the end of the trial and monitored for closure.

In group A, patients received three capsules of budesonide 3 mg once daily and in group B, patients received two capsules of budesonide 3 mg and one placebo capsule once daily.

At time of randomisation, prednisolone or methylprednisolone were accepted in a maximum dose of 20 and 16 mg/day, respectively, with a fixed tapering schedule within six weeks. Use of budesonide was permitted at the time of randomisation in a maximum dose of 9 mg/day, which had to be discontinued that day. Additional treatment with investigational agents, azathioprine, cholestyramine, cyclosporine or metronidazole, had to be stopped at least two weeks prior to randomisation. Furthermore, 5-aminosalicylates or other treatments for Crohn's disease, proton pump inhibitors and diuretics were not permitted during the trial after randomisation.

Trial design

The study was designed as a randomised, double-blind parallel group, multicentre clinical trial. One tertiary referral centre for inflammatory bowel disease and 32 regional centres (3 in Germany and 29 in the Netherlands) participated in the study. Eligible patients were randomly allocated to treatment with either budesonide 6 mg or 9 mg once daily for up to 52 weeks. Fixed outpatient visits were scheduled after 8, 24 and 52 weeks and additional visits were required in case of an increase in symptoms or adverse events (AEs). At each study visit, the CDAI was determined, and physical examination and laboratory tests were performed. All AEs, including signs and symptoms suggestive of corticosteroid-associated side effects, were recorded. Relapse was defined by a CDAI of more that 150 together with an increase of at least 60 points. If an increase in CDAI was likely explained by non-Crohn's disease causes, it was permitted to repeat the CDAI once and if it had normalised it was not considered to be a relapse. Time to relapse was defined as the time between the baseline visit and the first visit with a CDAI corresponding to a relapse.

Statistical analysis

Based on previous studies, we assumed an exponential disease-free survival and a relapse rate of 50% in the control group on budesonide 6 mg. We estimated that 95 patients per group were needed to detect an increase of at least 50% in time to relapse on budesonide 9 mg/day compared with 6 mg/day ($\alpha = 0.05$; $\beta = 0.20$). Patients who received at least one dose of study medication with at least one follow-up visit were included in the safety analysis and intention-to-treat (ITT) analysis. Kaplan-Meier estimates and log-rank tests of the survival distribution function were used for the analysis of the primary outcome measure time to relapse. The following covariates were included in the Kaplan-Meier analysis as strata: concomitant use of systemic corticosteroids at time of randomisation, disease location, smoking history, duration of disease, history of bowel resections, previous use of budesonide, and centre of inclusion. In the statistical analysis plan, 10, 15, 20 and 25% quantiles were used to obtain a 9 mg/6 mg ratio concerning time to relapse. Additionally, the median time to relapse (= 50% quantile) was estimated parametrically with SAS PROC LIFEREG assuming a Weibull distribution. The one-year relapse rates were analysed by Fisher's exact test. Baseline characteristics, secondary efficacy parameters and safety parameters were analysed by descriptive statistics. In case of missing values at the final examination, the last documented follow-up value was used. Results are given as mean ± standard deviation or median (range). The statistical evaluation was performed using SAS version 8.2.

Table 1. *Baseline characteristics according to study group*

	Budesonide 6 mg (n=76)	Budesonide 9 mg (n=81)
Sex (female/male)	47/29 (62/38)	45/36 (56/44)
Age (years)	35 (19-73)	35 (18-72)
Weight (kg)	69.5 (45-104)	74.9 (50-131)
Height (cm)	172 (150-198)	175 (154-194)
History of bowel resection	24 (32)	36 (44)
Smoking habits (never/ever)	30/46 (40/60)	35/46 (43/57)
Disease duration (years)	4.5 (0-32)	2.5 (0-49)
Disease involvement:		
• Ileum	72 (95)	77 (95)
• Caecum	42 (55)	41 (51)
• Ascending colon	24 (32)	24 (30)
• Transverse colon	15 (20)	18 (22)
• Descending colon	10 (13)	12 (15)
• Sigmoid colon	11 (15)	13 (16)
CDAI at study entry*	69 (-16-154)	76 (-41-165)
Medication (last 12 months):		
• Corticosteroids	33 (43)	28 (35)
• 5-Aminosalisylate	67 (88)	70 (86)
• Immunosuppressive	2 (3)	4 (5)

Values are given as median (range) or number (%). *In each treatment group the Crohn's Disease Activity Index (CDAI) was above 150 in one patient (protocol violation).

RESULTS

Patients

The recruitment was terminated after 160 patients had been included (22 in the tertiary referral centre), because of slow enrolment and because the observed overall relapse rate was far below the estimated rate. The enrolment period started in November 1997 and was discontinued in February 2001. Three patients were excluded from the ITT analysis and safety evaluation. One patient was lost to follow-up without any follow-up values, one patient did not take the study medication and one patient was randomised twice. Of the remaining 157 patients, 76 were assigned to the 6 mg/day group and 81 were assigned to the 9 mg/day group. The baseline characteristics did not differ significantly between the two treatment groups (*table 1*).

Early withdrawal from treatment

Out of 157 patients, 56 discontinued the trial prior to one year after baseline. The number of early terminations was equally distributed between the two treatment groups, 28 (37%) in the 6 mg/day group and 28 (35%) in the 9 mg/day group. A flow chart of study participation is shown in *figure 1*. In the 6 mg/day group, 17 out of 76 patients (22%) discontinued due to inadequate efficacy, 11 (14%) for other reasons and 48 (63%) completed the one-year follow-up in clinical remission. In the 9 mg/day group, 18 out of 81 patients (22%) discontinued due to inadequate efficacy, 10 (12%) for other reasons and 53 (65%) completed the one-year follow-up in clinical remission.

Efficacy: relapse-free survival

The relapse-free survival as a function of time is shown in *figure 2* for both treatment groups in the ITT analysis. By log-rank test, no statistically significant difference between the two groups was demonstrated (p=0.46). From the covariates included in the Kaplan-Meier analysis, concomitant use of corticosteroids at the time of randomisation was associated with the shortest time to relapse (p=0.03). No influence on time to relapse was shown by the covariates disease location, smoking history, duration of disease, history of bowel resections, previous use of budesonide and centre of inclusion. After one year, the probability of being relapse free was around 75% in both groups and the median time to relapse was far outside the observation interval and could not be estimated using nonparametric methods. The nonparametric Kaplan-Meier estimates of the 10% quantile were 124 days in the 6 mg

Figure 1. *Flowchart of patients during study participation*

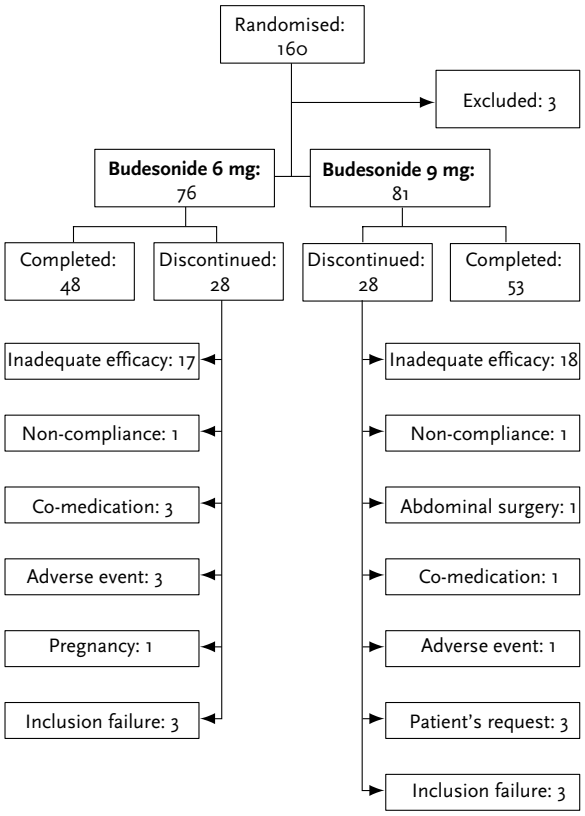
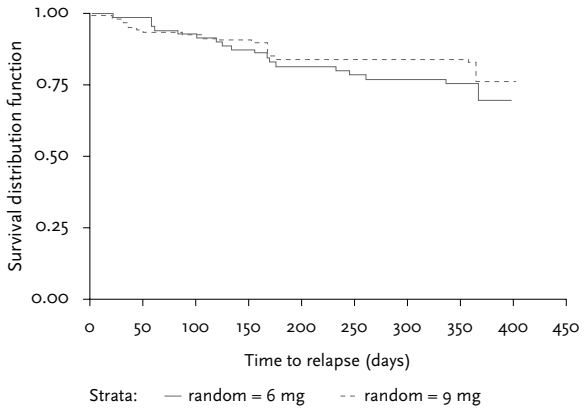


Figure 2. *Kaplan-Meier estimates of time to relapse with budesonide 6 mg/day and 9 mg/day in 157 patients with Crohn's disease in remission (ITT analysis)*



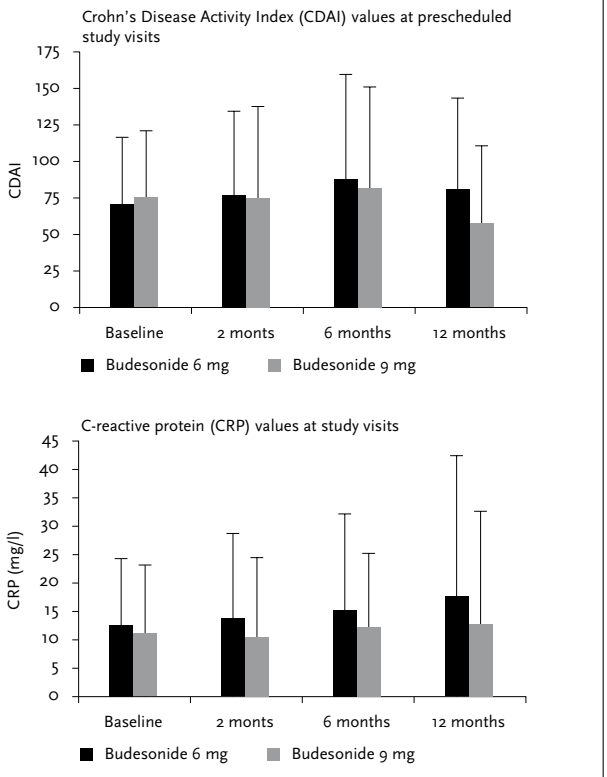
By log-rank test, no significant difference between the two treatment groups was observed (p=0.46). The hazards ratio with its 90% confidence interval is 0.773 (0.435-1.375).

group and 153 days in the 9 mg group, for the 15% quantile 165 days *vs* 175 days, and for the 20% quantile 232 *vs* 364 days, but the upper limit of the confidence interval was not estimable for the 20% quantile. The parametric estimates for the median time to relapse were 809 days (95% CI 360 to 1259) for the 6 mg group and 1049 days (95% CI 384 to 1713) for the 9 mg group. However, the maximal observation intervals in the study were 398 (6 mg group) and 402 days (9 mg group).

Efficacy: relapse rates

After the treatment period of one year, the relapse rate was not significantly different between the budesonide 6 and 9 mg groups (p=0.43). Relapse rates in the ITT were 24% (18/76) *vs* 19% (15/81) respectively and among those patients who completed the trial or discontinued due to a relapse 28% (18/65) *vs* 21% (15/71). The frequency of relapses was higher in the IBD referral centre compared with the non-IBD referral centres: 8 out of 22 patients (36%) and 25 out of 135 patients (19%), respectively. During the study period, the mean CDAI increased in the 6 mg/day group (from 71 ± 45 to 104 ± 86; not significant) and in the 9 mg/day group (from 76 ± 45 to 91 ± 84; not significant). Furthermore, C-reactive protein increased slightly in both groups from 12 ± 12 mg/l to 18 ± 23 mg/l in the 6 mg group and from 11 ± 12 mg/l to 15 ± 20 mg/l in the 9 mg group (*figure 3*). The erythrocyte sedimentation rate increased slightly in the 6 mg group from 16 ± 13 mm/h to 22 ± 1 mm/h, whereas it remained stable in the 9 mg group (15 ± 12 mm/h).

Figure 3. *The course of secondary efficacy parameters during maintenance treatment with budesonide*



Values represent mean ± SD.

Adverse events

In total, 195 AEs occurred in 101 patients during the study period. In the 6 mg group, 92 AEs occurred in 46 (61%) patients and in the 9 mg group, 103 AEs in 55 (68%) patients. An overview of all AEs classified by organ system is given in *table 2*. The intensity of the AEs was classified as mild or moderate in 94% of the cases. During the study period, no deaths occurred. A total of 12 serious adverse events (SAEs) were recorded in ten patients (*table 3*). The reason to classify these 12 events as serious was hospitalisation. None of these SAEs were clearly related to the study medication. Adverse events potentially related to corticosteroids are summarised in *table 4*. External steroid-related side effects such as acne, moon face and obesity were already present at baseline in 29% of the patients randomised to 6 mg/day and 22% of the patients in the 9 mg/day group. These initially present side effects resolved completely in nine patients (12%) in the 6 mg group and in six (7%) patients in the 9 mg group.

Table 2. *Summary of adverse events (numbers and %)*

Organ system	6 mg (n=76)	9 mg (n=81)
Endocrine	10 (13)	10 (12)
Eye	2 (3)	1 (1)
Gastrointestinal tract	9 (12)	11 (14)
Hepatobiliary tract	0	1 (1)
Infections	6 (8)	13 (16)
Injury, poisoning and procedure related	0	3 (4)
Metabolism and nutrition	4 (5)	9 (11)
Musculoskeletal and connective tissue	2 (3)	4 (5)
Nervous system	8 (11)	4 (5)
Pregnancy	2 (3)	0
Psychiatric	1 (1)	4 (5)
Renal and urinary tract	1 (1)	1 (1)
Reproduction and breast disorders	1 (1)	0
Skin and subcutaneous tissue	17 (22)	20 (25)
Surgery and medical procedures	1 (1)	0
Vascular	3 (4)	3 (4)
General disorders	5 (7)	5 (6)

DISCUSSION

The present trial was conducted to evaluate the time to relapse and one-year relapse rates, comparing 6 mg/day and 9 mg/day of budesonide in patients with quiescent Crohn's disease. Only 24% of patients treated with budesonide 6 mg/day relapsed within one year, compared with 19% of patients treated with 9 mg/day, without significant differences in time to relapse between both study groups. These relapse rates were lower than expected because in previous maintenance trials one-year relapse rates were over 50% in patients treated with budesonide 3 mg or 6 mg daily and placebo-treated controls.[16-18,20]

Although it was planned to include 190 patients in the present study, the recruitment was stopped after 160 patients for two reasons. First, due to the slow inclusion rate the study drug had reached its expiry date. And second, because of an overall (blinded) relapse rate far below the expected rates in the sample size calculation, it was highly unlikely that adding 30 more patients in this trial would result in different outcomes. Given 5% difference in relapse rates between both treatment groups in the ITT analysis, a much larger sample size would have been needed to reach

Table 4. *Summary of steroid-related adverse events*

	6 mg (n=76)	9 mg (n=81)
Acne	9 (12)	8 (10)
Moon face	6 (8)	9 (11)
Hirsutism	6 (8)	0
Headache	6 (8)	3 (4)
Abdominal pain	4 (5)	5 (6)
Obesity	4 (5)	7 (9)
Striae	2 (3)	4 (5)

Number of patients (%).

Table 3. *Serious adverse events during budesonide treatment*

Patient	Dose (mg)	Serious adverse event	Sex	Age (years)	Time (days)	Causal relation
1	6	Bartholini cyst surgery	F	21	34	Unlikely
2	6	Hypertensive crisis	M	67	354	Unrelated
3	6	Anal fistula	M	25	302	Unrelated
4	6	Extrauterine gravidity	F	34	290	Unrelated
5	9	Inguinal hernia	M	43	101	Unrelated
6	9	Vomiting, abdominal pain	M	22	164	Unlikely
7	9	Stab wound	M	23	62	Unrelated
8	9	Abdominal pain, diarrhoea	F	69	286	Unlikely
9	9	Psoas abscess	M	42	353	Unrelated
10	9	Ileus	M	34	141	Unlikely

F = female; M = male. Time is given in days from the start of the study medication to the first appearance of the serious adverse event.

significance, while the small difference of 5% between both groups may not be clinically relevant.

The magnitude of the difference in relapse rates in the present trial compared with previous placebo-controlled studies may have various explanations. First, the majority of patients (259 out of 270) in the studies by Greenberg, Löfberg, and Ferguson and colleagues were initially treated for active Crohn's disease with corticosteroids and randomised for the maintenance trials eight to 16 weeks after the onset of corticosteroid therapy if clinical remission was achieved.[16-18] In contrast, in the present study, clinical remission was induced by a variety of therapy modalities and the use of corticosteroids at the time of randomisation was associated with the shortest time to relapse. Therefore, this may partly explain the unexpected low relapse rate in the budesonide 6 mg group. The lower rate of relapses may also be explained by a potentially longer interval (3 to 18 months) between the onset of therapy for active Crohn's disease (i.e., treatment of last relapse) and inclusion in the present trial, compared with the previous maintenance trials. Due to the inclusion of patients with a longer disease-free period, selection of patients with a more benign course may have occurred, because the natural course of Crohn's disease may differ considerably between patients.[22] This potential selection bias was limited by excluding patients with an active disease-free period of more than 18 months. Finally, in the present study, the majority of patients were included by regional nonreferral IBD clinics. In these clinics, 25 out of 135 patients (19%) relapsed during the study period compared with eight out of 22 patients (36%) in the IBD referral centre. It may be so that patients treated in referral centres receive more aggressive disease and are more refractory to therapy.

Slow enrolment in the study was largely explained by the availability of azathioprine and methotrexate, which have proven efficacy in maintaining remission. If the patient was in remission on these immunosuppressants, it was considered unethical to discontinue them just for the study. Therefore, less than 5% of patients were on immunosuppressants in the year before randomisation, which were discontinued for intolerance in most of these cases.

After closure of recruitment in the present study, comparable low relapse rates were reported by Green and colleagues with budesonide 6 mg/day (19%) with a flexible dose between 3 and 9 mg/day (15%) in patients with Crohn's disease in remission.[23] A different definition of treatment failure was used, defined as moderate to severe symptoms over an eight-week period despite treatment with 6 mg in the fixed group and 9 mg in the flexible group, or a CDAI >200 with moderate to severe symptoms. Including a placebo group in the present trial would have solved the issue of the unexpected low relapse rates. Although scientifically justified, difficulties with patient recruitment were expected if a placebo group was included in the trial, because several maintenance modalities such as mesalamine, budesonide and azathioprine were widely available during the trial period.

In the present study, the frequency of adverse events was not different between the 6 mg/day and 9 mg/day treatment groups. In addition Greenberg and colleagues reported no significant differences in overall frequencies of adverse events within one year between groups treated with budesonide 3 mg/day (70%) or 6 mg/day (78%) or placebo (89%).[16] Focusing on potential corticosteroid-related events, no significant differences between placebo and budesonide 3 mg/day or 6 mg/day groups were observed in previous studies.[16-18] However, cortisol stimulation tests demonstrated mild adrenal suppression in the budesonide groups, compared with placebo.[16,18] In the present study, over 90% of the adverse events were of mild or moderate intensity and had usually resolved by the end of the study. None of the 12 serious adverse events had a probable relationship with the study medication. All these events were classified as serious because they required admission to hospital. The most frequently reported adverse events during the study period were likely related to corticosteroid treatment, such as acne, moon face and hirsutism. However, already at baseline, 29% of the patients receiving 6 mg/day and 22% of the patients receiving 9 mg/day showed external steroid-related side effects due to prior use of prednisone. Only 11% of patients in the 6 mg/day group and 15% in the 9 mg/day group developed corticosteroid-related side effects after baseline. Overall, the spectrum of adverse events during treatment with budesonide 6 mg/day or 9 mg/day is mild, which is in agreement with previous reports.

However, safety issues concerning osteoporosis due to long-term treatment with budesonide remain unanswered by this study. This is of importance as corticosteroids are considered to be an established risk factor for osteoporosis. As Crohn's disease in itself is also a risk factor for osteoporosis, the definite effects of corticosteroids on bone mineral density (BMD) remain less clear than initially thought.[24] Cino and colleagues reported a small but significant decrease in BMD over a two-year period in patients treated with budesonide compared with low-dose prednisone or nonsteroid therapy in a nonrandomised trial, which resulted in different phenotypes of Crohn's disease in the three cohorts.[25] In contrast, in a prospective randomised trial, Schoon and colleagues demonstrated significantly less loss in BMD during treatment with budesonide compared with prednisolone over a two-year period in corticosteroid-naive patients.[26]

In conclusion, a low relapse rate was achieved in patients with quiescent Crohn's disease, treated with budesonide 6 mg/day. No significant additional benefit was demonstrated by increasing the dose to 9 mg/day. On the other hand, the number of adverse events was similar in both treatment groups. In placebo-controlled trials, budesonide 6 mg daily

was able to postpone relapses, but was unable to prevent relapses in one year. Due to the absence of significant differences in the present trial, the efficacy of budesonide in prevention of relapses remains unproven. In individual cases, when dose escalation is needed, budesonide may be increased to 9 mg/day without a significant increase in adverse events over a one-year period.

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CASE REPORT

A patient treated with olanzapine developing diabetes de novo: proposal for hyperglycaemia screening

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ABSTRACT

We report a patient with schizophrenia who developed diabetes mellitus during treatment with olanzapine. The case confirms the pattern of atypical antipsychotic-related diabetic emergencies: rapid onset in relatively young patients, often with severe glucose derangements and serious complications. As diabetic emergencies have a high morbidity and mortality, regular glucose screening should be performed in patients with schizophrenia treated with atypical antipsychotics.

KEYWORDS

Atypical antipsychotics, diabetic ketoacidosis, hyperosmolar hyperglycaemic deterioration, olanzapine

INTRODUCTION

In the past decade, the prescription of the atypical antipsychotic drugs has been reported as a precipitating factor for diabetes mellitus (DM).[1] Diabetes in patients with schizophrenia treated with antipsychotics is usually classified as type 2 DM.[2] It is, however, often rapid in onset and presents as a syndrome with both characteristics of a hyperosmolar hyperglycaemic syndrome and diabetic ketoacidosis, with severe hyperglycaemia, hyperosmolarity, but also with ketoacidosis. We present a patient with schizophrenia, who developed DM *de novo* during treatment with the atypical antipsychotic drug olanzapine.

CASE REPORT

A 47-year-old male patient of African origin was brought into the emergency department of our hospital by ambulance because of a rapidly deteriorating level of consciousness, polydipsia and hypotension. The patient was referred from a psychiatric ward, where he had been treated for schizophrenia, paranoid type (DSM IV: 295.30), with olanzapine 15 mg/day for three months before admission.

On admission to the psychiatric ward three months earlier, the patient was violent and did not allow any invasive procedures. Olanzapine was started immediately. One month after his admission he agreed to blood glucose measurements. At that moment a nonfasting blood glucose was 9.6 mmol/l. Unfortunately, this elevated value did not reach the doctors' attention.

The patient had documented atopic rhinitis, for which he used a budesonide nose spray, and chronic bronchitis, for which he took a preventive dosage of doxycyline 100 mg/day and acetylcysteine 1200 mg/day.

Personal and family histories for diabetes mellitus were negative. Furthermore, he had no history of hypertension, dyslipidaemia, alcohol or drug abuse.

On physical examination he showed a decreased level of consciousness (EMV score 1-5-2), hypotension (RR 80/50 mm Hg) and decreased turgor. The patient's body mass index was 24.5 kg/m^2.

Laboratory analysis on admission (reference values between brackets) showed extreme hyperglycaemia of 118.7 mmol/l (<7.8), signs of severe dehydration (plasma creatinine 329 µmol/l (70-110)), serum sodium of 131 mmol/l (135-145), serum potassium of 4.3 mmol/l (3.5-5.0) and a slightly increased C-reactive protein of 19 mg/l (0-10). Blood gas

analysis showed metabolic acidosis: pH 7.07 (7.36-7.44), pCO_2 2.1 kPa (4.5-6.1), pO_2 12.7 kPa (10.0-14.0), HCO_3^- 4.5 (21-27), and anion gap 24 mmol/l (<12). Serum lactate was 2.25 mmol/l (0.63-2.43). Urine and blood cultures and chest X-ray showed no signs of infection. Unfortunately no urine analysis was performed for ketones.

The patient was admitted to the intensive care unit and treated with mechanical ventilatory support, with intravenous (iv) 0.65% sodium chloride, and iv insulin. During treatment he developed hypernatraemia and rhabdomyolysis (creatinine kinase 13410 U/l (0-200) on day 3).

After three days the patient could be detubated and after five days he could be transferred to the general ward where iv insulin was switched to subcutaneous (sc) insulin. Antipsychotic medication was switched from olanzapine to risperidone.

On hospital discharge he was using 28 units long-acting insulin combined with 16 units short-acting insulin three times a day. One month after discharge the insulin dose could be reduced to 16 units of long-acting insulin with 4 to 6 units short-acting insulin three times a day. Four months after discharge the sc insulin was replaced by metformin 1000 mg/day.

DISCUSSION

The patient described in this case report presented with severe life-threatening hyperosmolar hyperglycaemic deterioration of new-onset type 2 diabetes with characteristics of a ketoacidosis as well.

Such severe glucose disarrangements in such a short period in a patient who did not have documented diabetes mellitus, with a negative family history, without weight gain in the preceding period, and without the occurrence of other important precipitating factors for hyperosmolar deterioration such as infection, myocardial infarction, stroke, or excess alcohol consumption is very unusual for type 2 diabetes. After cessation of olanzapine, insulin dosages could be reduced rapidly, and the patient could eventually be started on oral antidiabetic agents.

The patient developed rhabdomyolysis. It was postulated that the hyperosmolar state inhibits the electrogenic sodium pump on muscle cells, impairing sodium-calcium transport, resulting in increased cytoplasmatic calcium levels which destroys muscle cells.[3]

Although several case reports describe the occurrence of diabetes mellitus with atypical antipsychotic use,[1] studies investigating relative risks of developing hyperglycaemia/DM in patients treated with olanzapine are not available. In a small study among 71 patients treated with olanzapine, 39% of these patients who did not have documented diabetes mellitus were hyperglycaemic (fasting plasma glucose >5.6 mmol/l).[4]

Several mechanisms have been proposed as to why olanzapine induces DM by influencing insulin secretion or insulin action. Firstly, olanzapine causes weight gain, thus increasing insulin resistance. However, as it has been shown that olanzapine can also induce diabetes without weight gain,[5,6] other mechanisms have to coexist. Both stimulating and inhibitory effects of atypical antipsychotics on insulin secretion have been mentioned.[7,8] The underlying molecular mechanisms are unknown. Clozapine, a related atypical antipsychotic, was found to inhibit insulin release through activation of K+ channels and thus hyperpolarisation of the cell membrane of pancreatic β-cells.[8] Suppression of compensatory insulin release has also been attributed to antagonism of muscarine receptors on the β-cells, as olanzapine is a potent nonselective muscarinic antagonist.[9]

Through similar mechanisms olanzapine might also act on cholinergic parasympathetic nerve endings to the liver, disrupting hepatic glucose metabolism.[9] In addition to these peripheral actions, certain atypical antipsychotics may affect central nervous system glucose regulation, either directly via the release of epinephrine and glucagon or via an effect on neural pathways to peripheral tissues.[9] Further research is necessary to unravel the molecular mechanism, as a better understanding of these mechanisms will influence the development of new agents without adverse metabolic effects.

Glucose monitoring is important in the prevention of hyperglycaemia/DM with antipsychotic treatment. A screening chart for hyperglycaemia in patients treated with atypical antipsychotics was proposed by the American Diabetes Association (*table 1*).[10] However, as glycaemic monitoring is considered impractical and costly,[11] it is often not practised. It has been shown that

Table 1. *Monitoring protocol for patients on atypical antipsychotics according to the American Diabetes Association (Diab Care 2004)*

	Baseline	4 weeks	8 weeks	12 weeks	Quarterly	Annually
Personal history	X					X
Weight (BMI)	X	X	X	X	X	
Waist circumference	X					X
Blood pressure	X			X		X
Fasting plasma glucose	X	*	*	X	*	X

*Represent additional measurements as proposed by the authors for the first year of treatment with olanzapine.

most of the newly diagnosed diabetes cases developed within the first months after starting olanzapine treatment,[1] and therefore it seems logical to monitor more closely in this period. Patients with (multiple) risk factors for DM (African origin,[1] obesity, family history) should be checked even more closely.[12] Older age should not be regarded as a major risk factor, since it was found that a striking 75 to 91% of patients with schizophrenia during antipsychotic treatment developed diabetes before the age of 50 years.[13] We therefore advise monitoring glucose at monthly intervals in all patients, irrespective of age, during the most risky phase, i.e. the first three months of treatment with olanzapine. Then, quarterly monitoring is advised for the first year (*table 1*), after which we advise once yearly monitoring. Future research has to prove whether these monitoring intervals are strict enough to prevent diabetic emergencies.

As the various atypical antipsychotics differ in their potential for causing glucose dysregulation, with olanzapine having greater adverse effects on glucose levels than risperidone, quetiapine, and perfenazine, we advise switching to a one of these less adverse agents if disarrangements in glucose metabolism occur.[14] In our patient the insulin dose could soon be decreased by using risperidone and eventually the insulin was replaced by oral antidiabetic agents.

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CASE REPORT

Verapamil-induced erythermalgia

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ABSTRACT

Erythermalgia is a rare clinical syndrome characterised by intermittent, usually symmetrical burning pain, warmth and dermal erythema of the extremities with an amelioration of discomfort by cooling of the extremity. In this report, we describe a patient with erythermalgia caused by long-term verapamil use. After discontinuing the verapamil, the symptoms improved dramatically within two weeks.

KEYWORDS

Calcium antagonists, erythermalgia, erythromelalgia, verapamil

INTRODUCTION

Erythromelalgia and erythermalgia are rare disorders characterised by severe burning pain, warmth and redness of the extremities. Symptoms are commonly exacerbated by exposure to heat and improved by cooling of the extremity. The term erythromelalgia derived from the Greek words erythros (red), melos (extremity) and algos (pain) was introduced by Mitchell.[1] Smith and Allen have suggested changing the name to erythermalgia in order to emphasise the importance of the increased skin temperature (thermé).[2] Since then, these terms are used indiscriminately as synonyms, which may cause confusion. At present two competing classifications exist. In the classification by Kurzrock *et al.* patients are categorised into early-onset erythromelalgia and adult-onset erythromelalgia (secondary or idiopathic forms).[3] Early-onset erythromelalgia manifests in childhood or during adolescence and often shows a familial occurrence. Adult-onset erythromelalgia may be associated with a myeloproliferative disorder such as thrombocythaemia, as well as with other diseases and several drugs. On the other hand, Michiels *et al.* distinguish three categories: erythromelalgia, and primary and secondary erythermalgia.[4,5] Erythromelalgia is aspirin sensitive and invariably associated with thrombocythaemia. Erythromelalgia is usually asymmetrical, whereas erythermalgia is symmetrical. Primary erythermalgia occurs spontaneously in childhood or adolescence in the absence of any detectable underlying disorder. Secondary erythermalgia originates from associated disorders or develops as a consequence of side effects of drugs. We describe a patient with secondary erythermalgia due to long-term treatment with verapamil.

CASE REPORT

In May 2002, a 66-year-old man developed recurrent attacks of redness, swelling and painful burning sensations of both feet, occurring particularly in a warm environment and during evenings. Attacks lasted several hours and the unbearable burning pain was relieved only by immersion of his feet in ice cold water. Symptoms were particularly provoked by exertion, eventually limiting his walking distance to 10 m. As a result, he was practically housebound. He reported no fever, arthralgia or increased bleeding tendency. His history included chronic atrial fibrillation. Physical examination showed red, warm, oedematous feet with intact peripheral arterial pulsations (*figure 1*). His pulse was irregular with a frequency of 70 beats/min. During a period of 14 months, he was evaluated by many specialists, including vascular surgeons, rheumatologists, internists, neurologists and pain specialists. Extensive laboratory tests showed no abnormalities. Ankle-brachial pressure index and angiography of the legs were normal. Electromyography showed no signs of polyneuropathy. Numerous pain killers such as high-dose aspirin, nonsteroidal anti-inflammatory drugs, COX-2 antagonists and morphinomimetics, as well as acenocoumarol and clopidrogel, were tried without any improvement in his symptoms (*figure 2*). Transdermal

Figure 1. *Bilateral erythromelalgia with red, warm oedematous feet*

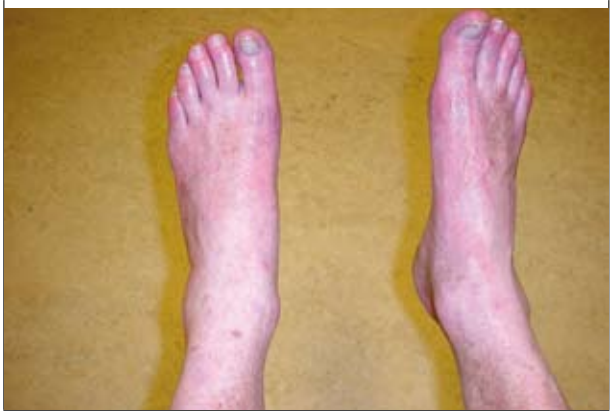

Figure 2. *Bag full of pills with which the patient presented*

electric nerve stimulations (TENS) and various homeopathic drugs were not helpful either. Chemical symphathectomy, performed three and 12 months after his first presentation, only aggravated his symptoms. The symptoms gradually worsened and were eventually present constantly throughout the day and night, making normal life almost impossible.

In July 2003, he was referred to our department. At this time, he was taking gabapentin, various homeopathic drugs and using a TENS device to reduce his pain. We established a diagnosis of erythermalgia. Evaluation of all the previously performed tests did not reveal an underlying cause for erythermalgia. However, our attention was drawn to the fact that he had been taking long-acting verapamil (Isoptin SR 120 mg/day) for the last six years for his chronic atrial fibrillation. After consulting his cardiologists we stopped the verapamil and started digoxin. Within two weeks, his complaints improved dramatically and at this moment, three years later, he is completely free of symptoms.

DISCUSSION

Drug-induced erythermalgia has been described in association with long-term use of ergot derivatives such as bromocriptine and pergolide.[6] Five case reports have reported erythermalgia caused by calcium antagonists (*table 1*). To the best of our knowledge, this is the second report of erythermalgia as a side effect of verapamil. The long time interval (five years) between the first dose of verapamil and the occurrence of erythermalgia, as described in this case, has not been reported before. Erythermalgia secondary to drugs develops insidiously and disappears within a few weeks after discontinuation. Our patient, understandably, declined a rechallenge, but rapid regression of symptoms immediately after withdrawal strongly suggests a causal relationship. Verapamil is a widely used drug and is indicated for the treatment of hypertension, cardiac arrhythmias and angina pectoris. It is a phenylalkylamine derivative which inhibits the slow inward current of calcium ions across the cell membrane of

Table 1. *Case reports on calcium antagonists-induced erythromelalgia*

Year	Author	Drug	Dose (mg/day)	T1	T2
1983	Brodmerkel[7]	Nifedipine	40	NS	Immediate
1983	Fisher *et al.*[8]	Nifedipine	60	8 weeks	2 days
1989	Levesque and Moore[9]	Nicardipine	60	3-4 weeks	'Rapidly'
1992	Drenth *et al.*[10]	Verapamil	120	Few months	1-2 weeks
1996	Sunahara *et al.*[11]	Nifedipine	40	1 day	24 hours
2007	Nanayakkara *et al.* Current report	Verapamil	120	5 years	2 weeks

T1 = time between first dose and onset of erythromelalgia; T2 = time from discontinuation of the calcium antagonist to resolution of erythromelalgia; NS = not specified.

smooth muscles, thereby causing coronary and peripheral vasodilatation. The mechanism of verapamil-induced erythermalgia is unknown, but presumably is related to its vasodilatory action. Interestingly, nifidepine and diltiazem have been used successfully for the treatment of erythromelalgia.[12]

The underlying mechanism of erythermalgia is not known. Recently the origin of primary erythermalgia (or early-onset erythromelalgia) has been revealed.[13] This autosomal dominant disorder is a neuropathic disorder and may be caused by a mutation in SCN9A, the gene that encodes the Nav1.7 voltage-gated sodium channel which is predominately expressed in sensory and sympathetic neurons. Although this mutation causes membrane depolarisation in both types of neurons, it causes hyperexcitability in sensory neurons and hypoexcitability in sympathetic neurons.[14]

Nevertheless, it is generally believed that erythermalgia is primarily a vascular disorder which may be caused by an increased vasodilatation of the microvasculature in the extremities.[15] In general, vascular tone is regulated by many factors such as nitric oxide and other vasoactive substances, sympathetic and parasympathetic nervous system. Disturbance of one of these factors could deregulate the vascular tone. It may be argued that vasodilatation in patients with inherited erythermalgia may be induced by neuronal deregulation in two ways. Firstly, a decreased stimulation of sympathetic neurons may cause vasodilatation. Secondly, stimulated sensory neurons can induce neurogenic dilatation which is mediated by calcitonin gene-related peptide (CGRP).[16] Therefore, hyperexcitability of sensory neurons may also increase neurogenic dilatation.

Drugs such as calcium antagonists are also known to induce vasodilatation by inhibiting Ca^{++} influx into vascular smooth muscle. This vasodilatation may have played a decisive role in the induction of symptoms of erythermalgia in our patient. Presumably, the chemical sympathectomy enhanced vasodilatation[17] and microvascular shunting, causing aggravation of symptoms.

Erythermalgia or erythromelalgia, although rare, is an important diagnostic consideration not only because erythromelalgia sometimes responds to aspirin but also to avoid potentially harmful treatment attempts provoked by excruciating pain. If left untreated erythromelalgia associated with myeloproliferative disorders may progress towards painful acrocyanosis and even peripheral gangrene.[18] Erythermalgia could resemble cellulitis, thrombophlebitis and other vaso-occlusive and inflammatory disorders. However, recognition of erythermalgia in fact is not difficult given the characteristic response of pain to cold.

As illustrated in this case, many doctors are not aware of this rare clinical syndrome. During a period of 14 months, our patient visited many specialists and underwent extensive diagnostic procedures and therapies, including invasive procedures such as chemical symphathectomy. Several drugs were added to the patient's medication without any improvement of symptoms. Conversely, the symptoms subsided after discontinuing verapamil. An important general lesson to be learned is to pay attention to a patient's medication before performing extensive diagnostic and therapeutic procedures. It should be appreciated that discontinuation of medication can also be used as a simple diagnostic test for clinical syndromes, such as erythermalgia.

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CASE REPORT

Brucellosis, an uncommon and frequently delayed diagnosis

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ABSTRACT

In the Netherlands, brucellosis is uncommon. Diagnosis is difficult and frequently delayed. We present three patients with back pain and/or arthralgia caused by brucellosis. We emphasise the importance of considering brucellosis in patients returning from a stay in a rural area of an endemic country, who present with osteoarticular symptoms and signs of chronic inflammation. Clues to the diagnosis come from a thorough medical history.

KEYWORDS

Brucellosis, spondylodiscitis, zoonotic infection

INTRODUCTION

Brucellosis is the most common zoonotic infection worldwide.[1,2] It is endemic in the Mediterranean region, the Middle East, Latin America and parts of Asia and Africa, but the epidemiology is changing over the last decades due to socioeconomic changes, improved disease recognition and eradication programmes.[2,3]

In the Netherlands brucellosis is uncommon.[4] The presentation of an infection caused by *Brucella* species can be extremely variable and nonspecific and diagnosis is therefore difficult. Early recognition is important, as delay in treatment allows disease progression resulting in complications. The following cases illustrate the importance of considering brucellosis in patients with back pain or arthralgia together with signs of chronic inflammation, particularly when they have a history of travel to rural areas of countries where brucellosis is endemic.

CASE REPORTS

Patient A, a 72-year-old Turkish male, presented to the emergency department with severe lower back pain. Acute lumbago was diagnosed, nonsteroidal anti-inflammatory drugs were prescribed and the next day he was discharged. However, in the next two weeks, the pain increased and he was readmitted. His medical history, with the help of a translator, revealed that the pain had started several weeks before admission and was accompanied by fatigue, malaise and a weight loss of 12 kg without any fever. Three months earlier, the patient had stayed with relatives in Turkey where he consumed local meat. Physical examination revealed a systolic cardiac murmur. Local tenderness to gentle spinal percussion was elicited and neurological examination was unremarkable. The temperature was normal. Laboratory studies revealed an erythrocyte sedimentation rate (ESR) of 98 mm/h (0-20), a haemoglobin (Hb) concentration of 6.2 mmol/l (8.5-10), a normal white blood cell count (WBC) and a C-reactive protein level (CRP) of 57 mg/l (0-9). Lumbosacral spine radiographs showed joint space narrowing at L4-L5 and L5-S1. On the suspicion of spondylodiscitis, magnetic resonance imaging (MRI) was performed. It showed reactive changes at L4-L5 and L5-S1, consistent with severe discopathy. A cardiac ultrasound showed sclerosis of the aortic valve only. Both pairs of blood cultures grew *Brucella melitensis* after five days. The MRI scan was re-evaluated and early spondylodiscitis was considered. *Brucella* spondylodiscitis with bacteraemia was concluded and the patient was treated with rifampicin 600 mg/day and ciprofloxacin 750 mg twice daily orally for three months. After one week the pain had considerably decreased and after three weeks he was free of symptoms. ESR, CRP and Hb levels had improved. The patient has been well during a follow-up of seven months.

Patient B, a 28-year-old Indian male, residing in the Netherlands, presented with a three-month history of fever, progressive lower back pain and a weight loss of 12 kg. Eight months earlier, he had stayed with relatives in rural India. The patient had repeatedly visited his general practitioner with these symptoms and laboratory results showed signs of inflammation, but a diagnosis had not been made. On admission, there was local tenderness on percussion of the lumbosacral spine. Neurological examination showed a positive Lasègue´s sign and a diminished Achilles tendon reflex on the left side. The temperature was 37.8°C. Laboratory results revealed an ESR of 79 mm/h, a CRP of 89 mg/l, an Hb concentration of 7.5 mmol/l and a normal WBC. MRI of the lumbosacral spine showed findings consistent with spondylodiscitis of L4-L5 with epidural and presacral abscess formation (*figure 1*). Because of the neurological dysfunction, lumbar spinal surgery was performed. During the procedure, pus was removed from the epidural space and sent for microbiological examination. After four days, cultures grew *Brucella melitensis*. At that time, a *Brucella* agglutination test on serum was positive at a titre of 1:320. Three pairs of blood cultures remained negative. The patient was successfully treated with ciprofloxacin 750 mg twice daily and doxycycline 100 mg twice daily orally for three months. After three weeks, the pain had diminished and the ESR, CRP and Hb concentration had improved. During a follow-up of four years, there had been no signs of relapse.

Patient C, a 51-year-old Turkish female, living in the Netherlands, presented to the emergency department with dyspnoea, a productive cough and fever. She had a previous medical history of chronic obstructive pulmonary disease (COPD). On physical examination, the temperature was 37.9°C and the peripheral oxygen saturation was 98%. Auscultation revealed expiratory wheezing and no cardiac murmurs. Laboratory workup showed an Hb concentration of 7.2 mmol/l, a normal WBC and a CRP of 61 mg/l. The ESR was not determined. The chest X-ray showed no infiltrates. COPD exacerbation due to an upper respiratory tract infection was diagnosed. She was admitted and treated with corticosteroids and a bronchodilator. The day after, she developed high fever and blood cultures were taken. On the suspicion of pneumonia, antibiotics were started. After five days, six pairs of blood cultures grew *Brucella melitensis*. At that time, medical history revealed that she had been experiencing fatigue, malaise, back pain and arthralgia for several weeks before admission. Moreover, she had recently stayed in Turkey with relatives

Figure 1A. *On T1-weighted images, decreased signal intensity of the vertebral bodies L5 and S1 is seen with hypointense masses in paraspinal soft tissues and in the epidural space*

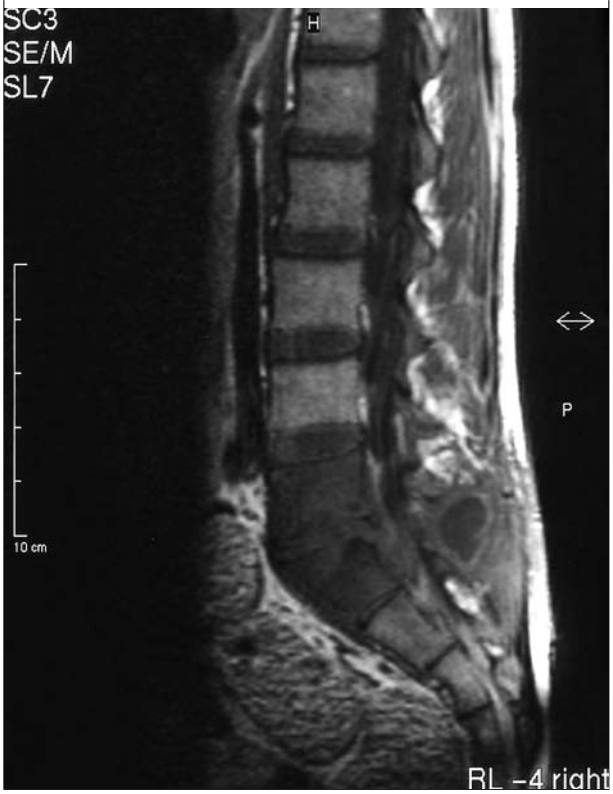
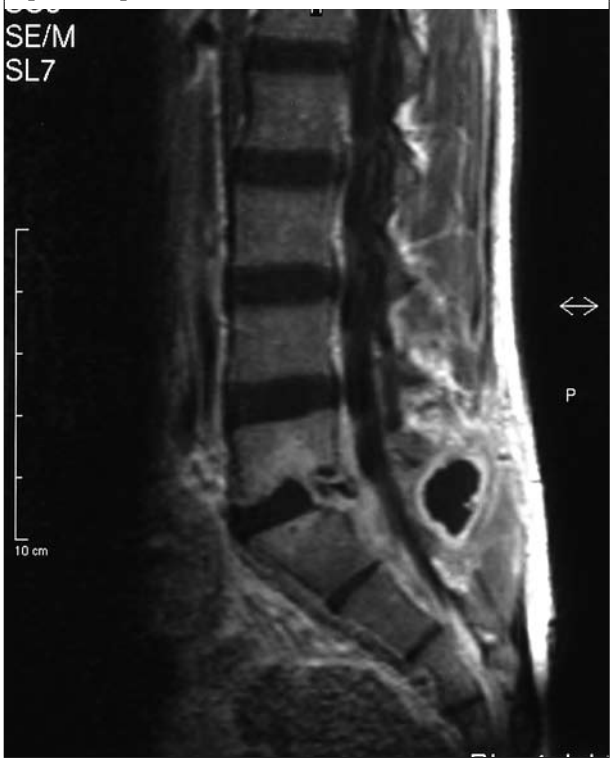


Figure 1B. *Contrast-enhanced T1-weighted images show enhancement of the inferior endplate of L5 and the superior endplate of S1, the disc space at L5-S1 and the masses in paraspinal soft tissues and in the epidural space.*



where she had consumed local cheese. Lumbosacral MRI was normal. The patient was treated for *Brucella* bacteraemia without a primary focus with rifampicin 600 mg/day and doxycycline 100 mg twice daily orally for six weeks. After one day, the fever had disappeared and she was discharged. During one year of follow-up, there were no signs of relapse.

DISCUSSION

Brucellosis is a zoonotic infection caused by small gram-negative coccobacilli of the genus *Brucella*. Four species are known to cause human disease, each having their own specific animal host: *B. melitensis* (goat, sheep, camel), *B. suis* (pig), *B. abortus* (cattle), and *B. canis* (dog).[2] Transmission occurs through cuts and abrasions of the skin, via the conjunctiva, by inhalation of infected aerosols or by consumption of contaminated food (unpasteurised dairy products, raw meat).[2] Brucellosis can be occupational, e.g. in veterinarians, farmers and laboratory workers.[2] In our cases, brucellosis was probably caused by ingestion of contaminated food.

Brucellosis is a systemic infection with a wide clinical spectrum and symptoms are often nonspecific. The most common symptom is fever and brucellosis is a well-documented cause of fever of unknown origin. Other prominent symptoms are sweats, fatigue, malaise, anorexia, arthralgia and weight loss. Because brucellosis is rare in the Netherlands, it is often not recognised. Our three cases demonstrate the importance of taking a thorough patient history, including questions about recent travel to countries of origin in non-native patients. Due to incomplete medical history taking, there was a delay in making the diagnosis in all three patients. The combination of increasing lumbar pain and/or arthralgia and signs of chronic inflammation together with their visit of an endemic country and the consumption of potentially contaminated food should have raised the suspicion of brucellosis with or without accompanying spondylodiscitis.

Although virtually any organ system can be affected, osteoarticular involvement is the most frequent complication of brucellosis.[2] Other, less frequent complications are epididymoorchitis, meningitis and endocarditis.[5-7] Two out of our three cases had proven osteoarticular involvement. Patient C had arthralgia only.

Physical examination is usually normal and even fever can be absent, which is illustrated by patients A and B. Laboratory results are nondiagnostic as well. WBCs are usually normal to low and pancytopenia can occur. ESR and CRP are usually elevated. Subtle elevation of liver enzymes frequently occurs.[2] Radiological examination can be helpful in identifying focal disease. MRI is the most suitable modality for early detection of abnormalities.[8-10] Even with MRI, however, the diagnosis of discitis may be difficult, which is demonstrated in case A.

Confirmation of brucellosis requires isolation of the organism from blood or tissue. The sensitivity of blood culture ranges from 15 to 80%, due to differences in laboratory techniques.[2] Using conventional culture methods, *Brucella* bacteria tend to grow slowly and cultures generally become positive after several weeks. Culture in a biphasic medium, lysis concentration and automated culture systems, such as BACTEC™, have been recommended to improve the recovery of *Brucella* spp. However, these methods are not routinely used in all laboratories. Therefore, when brucellosis is suspected, the clinician should communicate with the medical microbiologist to hold cultures for several weeks, to perform blind subcultures, or to use specific culture methods to prevent false-negative results. Additionally, knowledge of a potential *Brucella* infection may prevent airborne spread and subsequent contamination of laboratory personnel.[2,11] Awaiting the results of blood cultures, serological testing can be done. The serum agglutination test is the most commonly used. Titres above 1:160 in the presence of a compatible clinical picture are considered diagnostic. In patient B, serology would have been diagnostic in the absence of positive blood cultures.

The treatment of brucellosis has been extensively studied. The antibiotic regimens proposed by the World Health Organisation (WHO) in 1986 are still considered the gold standard. They consist of doxycycline 100 mg twice daily orally for six weeks with either rifampicin 600-900 mg/day orally for six weeks or streptomycin 1 g/day intramuscularly for two to three weeks.[12] Several alternative regimens have been proposed. Replacing streptomycin by gentamicin is considered equally effective.[13,14] Quinolones have been frequently studied but their role is still controversial. Clinical studies were small and major differences in design hamper comparison.[15] Overall, there seems to be a lack of evidence supporting the inclusion of quinolones in the initial therapeutic regimen.[16] A recent report on spinal brucellosis shows equal efficacy of quinolones and rifampicin compared with the classical doxycycline and streptomycin combination. However, the higher costs make this regimen unattractive as first-choice therapy in developing countries.[17] Finally, triple therapy adding an aminoglycoside or cotrimoxazole to the standard regimen has been associated with a lower relapse rate, but is not routinely used yet.[18,19] Prolonged treatment seems to be advisable when complications such as spondylodiscitis occur.[18,20] Patient A and B, who had osteoarticular complications, received prolonged treatment combining rifampicin and ciprofloxacin without signs of relapse. Patient C, who suffered from systemic brucellosis, was treated with doxycycline and rifampicin during the standard recommended period of six weeks.

CONCLUSION

Although brucellosis is rare in the Netherlands, it is important to consider the diagnosis when patients returning from native endemic countries present with a clinical picture of arthralgia or back pain and chronic inflammation. A thorough medical history is of paramount importance, because it provides clues to the diagnosis.

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LETTER TO THE EDITOR

Oxybutynin for hyperhidrosis

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Dear Sir,

Mijnhout *et al*. reported a case of a 56-year-old woman with severe hyperhidrosis, who was successfully treated with oxybutynin, in the October 2006 issue of this journal.[1] We confirm the effectiveness of oxybutynin for hyperhidrosis in two patients and report the results of one patient who was treated with glycopyrronium bromide, another parasympathicolytic drug.

Patient A was a 26-year-old man who was referred to our outpatient clinic because of hyperhidrosis of his whole body - except his hands - following a thoracic sympathectomy in December 2004 for bilateral hyperhidrosis palmaris. He had to change his clothes several times during the day and night. Also, he had a depressive mood with anhedonism, loss of appetite and a disrupted sleep pattern. No cause of hyperhidrosis was found after extended evaluation. It was concluded that the hyperhidrosis was compensatory to the sympathectomy, as has been reported in combination with depression.[2] With 2.5 mg oxybutynin three times a day, his sweating decreased substantially. However, after increasing the dose to 5 mg three times a day, he had difficulty in urinating. We switched to glycopyrronium bromide 3 mg/day and later twice daily after informed consent from the patient. The glycopyrronium bromide 3 mg tablets were compounded by the pharmacy department of the University Medical Centre Groningen. Glycopyrronium bromide (1,1-Dimethyl-3-(a-cyclopenyl)mandelyloxypyrrolidium bromide) is an anticholinergic drug registered in the USA for treatment of peptic ulcer disease, as anaesthetic premedication to reduce salivary secretion, as protection against adverse effects of cholinergic agents and for treatment of vagal reflex associated cardiac arrhythmias. Unfortunately, the patient experienced little effect. The oxybutynin was restarted.

Patient B was a 16-year-old woman with generalised hyperhidrosis without underlying disease, although she had Raynaud's phenomenon, and an increased level of plasma antinuclear antibodies (ANA) in 2004, which was discovered during evaluation of the Raynaud's disease. She had become socially isolated and depressed. With 2.5 mg oxybutynin three times a day, she was satisfactorily treated with no side effects.

In conclusion, we support the statement by Mijnhout *et al*. that oxybutynin merits consideration in patients with idiopathic hyperhidrosis. Hyperhidrosis may cause severe psychological and social impairment and demands the doctor's attention.

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Netherlands
The Journal of Medicine

PHOTO QUIZ

Fever and cough in a patient with diabetes

J.A. Ropela, L. van Die, W.J.G. Oyen, G.A. Rongen*

Departments of General Internal Medicine, Radiology and Nuclear Medicine, Radboud University Nijmegen Medical Centre, *corresponding author

CASE REPORT

A 76-year-old woman with diabetes mellitus type 2, coronary artery disease with atrial fibrillation and chronic microcytic anaemia presented to the emergency room with fever and cough for one week. Five years prior to presentation, she had been evaluated for pleural effusion after complicated coronary artery bypass graft (CABG) surgery without a definite diagnosis. In subsequent years she was repeatedly admitted to the hospital for evaluation of intestinal blood loss, diagnosed as diverticular bleeding while treated with oral anticoagulation. A few months prior to admission, she was diagnosed with an anxiety disorder and depression with cognitive impairment.

Her medication comprised atenolol, furosemide, triamterene, amiodarone, aspirin, domperidone, tolbutamide, insulin, isosobide mononitrate, venlafaxine, phenprocoumon, paracetamol and iron suppletion.

On admission we saw an ill patient with fever (39°C). Examination of her chest revealed dull percussion and reduced breathing sounds over the lower lobe of the right lung. A blood culture revealed *Salmonella typhimurium*. A chest X ray was performed and compared with a previous X-ray (see *figures 1* and *2*).

Figure 1. *Chest X-ray (June 2005)*

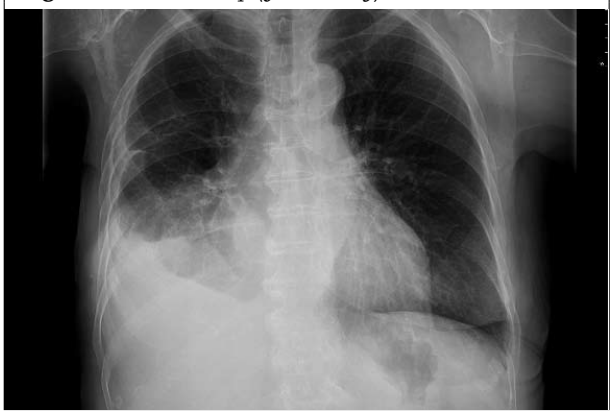
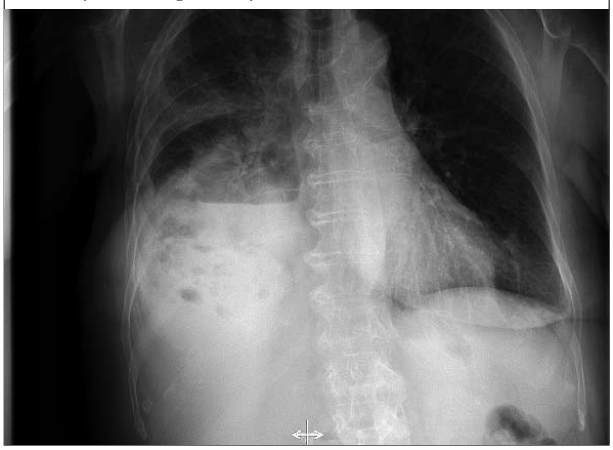


Figure 2. *Chest X-ray at presentation on emergency ward (February 2006)*



WHAT IS YOUR DIAGNOSIS?

See page 358 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (ON PAGE 357)

FEVER AND COUGH IN A PATIENT WITH DIABETES

DIAGNOSIS

The chest X-ray on admission shows an increase in the pleural effusion and several air-fluid interfaces.

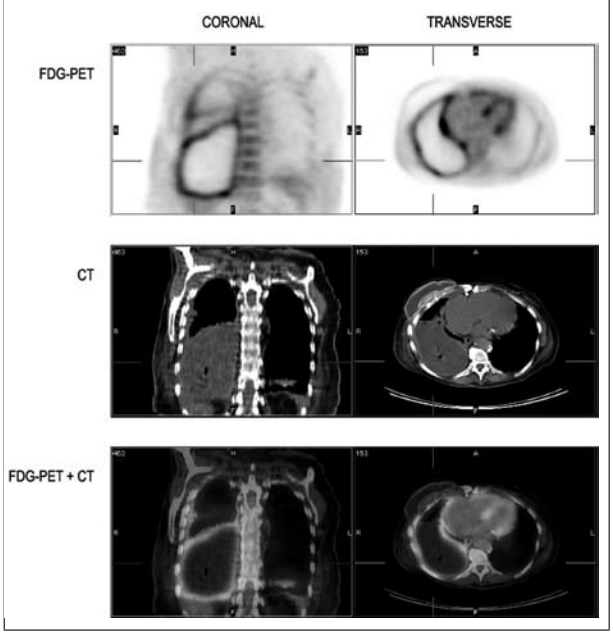
Our working diagnosis was either a partitioned empyema with local gas formation and/or intrapulmonary abscesses with either gas-forming bacteria or connection to the bronchus.

To confirm this diagnosis, a pleural puncture was performed. *Salmonella typhimurium* was cultured from the exsudate. An FDG-PET-CT was performed to identify the source or other localisations of infection. FDG-PET-CT revealed inflammation of the pleura of the whole right lung (*figure 3*).

Our diagnosis is empyema caused by *S. typhimurium*. *S. typhimurium* is able to form gas.[1]

The source of this infection was not elucidated. FDG-PET-CT scan did not reveal any other focus of infection. This frail woman was treated with ciprofloxacin. Thoracic drainage was not performed because of presumed risk of pneumothorax associated with pulmonary involvement in this infection. Thoracic surgery was not performed because of her poor general condition and comorbidity. Her fever as well as the CRP normalised within three weeks. She was discharged to a nursing home.

Figure 3. *FDG, PET and CT revealing significant glucose uptake of the pleurae of the right lung*



REFERENCE

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Impact factor of *the Netherlands Journal of Medicine* >1!

The impact factor (IF) is an important measure to estimate the scientific standard and ranking of a biomedical journal in the international field. In 2001, the former editor had already expressed his hope that the IF would exceed the level of 1.[1] Since we took over the editorship of *the Netherlands Journal of Medicine* in 2002, we were faced with a change in publisher and a drop in the IF (*see figure 1*). We have worked hard to improve the standard of the journal, made the journal freely accessible on-line and introduced an on-line submission system in 2005.[2,3] This has helped to make the journal more attractive and broaden its spectrum. We are proud to announce that our efforts have finally resulted in achieving the milestone of an IF >1.0 this year, for the first time in the journal's 50-year history!

Figure 1. *ISI impact factor of the Netherlands Journal of Medicine 1992-2006*

Anton Stalenhoef
Editor-in-Chief of the Netherlands Journal of Medicine

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The Netherlands Journal of Medicine: 50 years old

In a meeting on 9 November 1957, the Nederlandsche Internisten Vereeniging (NIV; the Netherlands Association of Internal Medicine) decided to start a scientific journal for internists, which was called *Folia Medica Neerlandica* (FMN). During the first years the FMN was actively supported by the *Vereeniging Nederlandsch Tijdschrift voor Geneeskunde*. At that time, there was ample discussion about the use of English as the language of the journal, but the conclusion was that it was one step too far at that moment.

In February 1958 the first issue came out and it was planned to start as a quarterly journal. The first editorial board consisted of Dr Lindeboom as editor, assisted by Drs Drukker, Lopes Cardozo, Majoor and Pannekoek. A few months later Dr Zuidema joined the board. At the beginning, there was support from the USA by Drs Snapper, Van Eck and Heinemann, from Israel by Dr Groen and from Curacao by Dr Van der Sar. In 1973 the name FMN was changed into *the Netherlands Journal of Medicine*.

The current editors intend to give special attention to the 50th birthday of the journal. We have invited our predecessors to write impressions about the history of the journal, which we will publish in the course of 2008.

The current editors thank all persons, authors and reviewers, who have contributed to the journal during the past 50 years. We congratulate the NIV administration and all the readers with such an old and at the same time such a young and vital journal.

Theo Thien
Associate editor of the Netherlands Journal of Medicine

PHOTO QUIZ

A patient with acute renal failure

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CASE REPORT

A 78-year-old female was admitted with renal insufficiency. She presented with dyspnoea on exertion and complained of painful and stiff hands. Photographs of her hands *(figure 1)* and face (*figure 2)* are shown. Blood pressure was 150/95 mmHg.

Laboratory tests showed an ESR 29 mm/h, WBC count 13.8 G/l, haemoglobin 8.3 mmol/l (nadir 6.3), platelet count 177 G/l (nadir 96), sodium 141 mmol/l, urea 10.8 mmol/l, creatinine 144 µmol/l (zenith 299), potassium 3.5 mmol/l, lactate dehydrogenase 1162 U/l (zenith 1500) and plasma renin activity (PRA) 7270 fmol/l/sec (zenith 9950).

ANA was ≥1:640, anti-dsDNA and anti-centromere antibodies were negative but anti-Scl70 was positive. Urinalysis showed protein 1.1 g/l, leucocytes 0-5 (high power fields; HPF) and erythrocytes 40-50 (HPF).

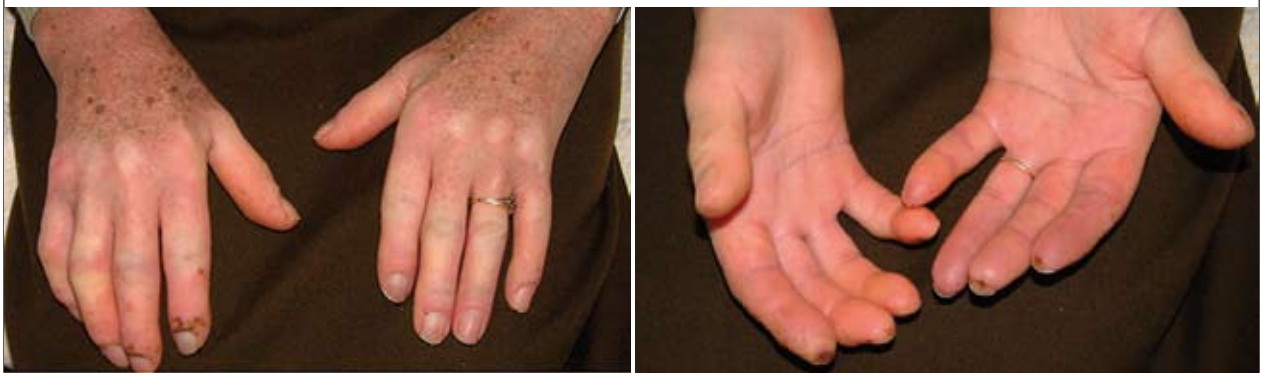
WHAT IS YOUR DIAGNOSIS?

See page 361 for the answer to this photo quiz.

Figure 2. *Patient's face*

The patient has consented to the use of her pictures.

Figure 1. *Patient's hands*



ANSWER TO PHOTO QUIZ (ON PAGE 360)
A PATIENT WITH ACUTE RENAL FAILURE

DIAGNOSIS

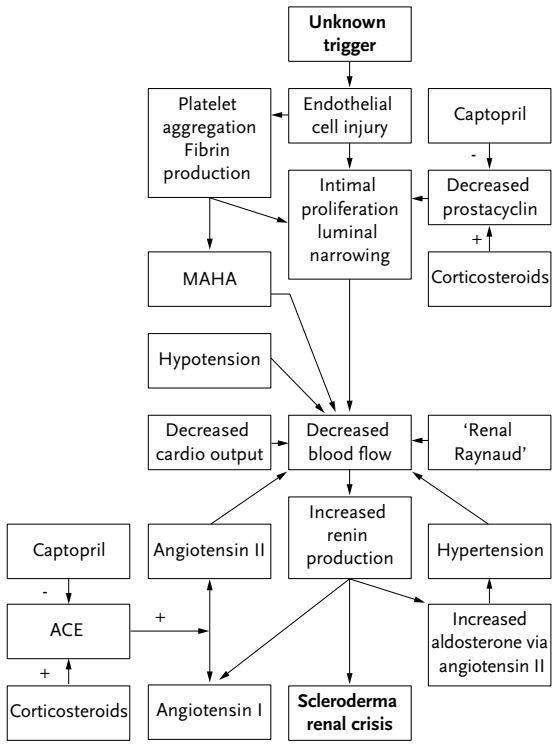
The patient was diagnosed with diffuse cutaneous systemic sclerosis three years ago. There was evidence of cardiac and pulmonary involvement. She was treated with prednisone 15 mg/day besides methotrexate, acenocoumarol, pantoprazole, folic acid, furosemide and carbasalate calcium. She now presented with a scleroderma crisis (SRC), with renal failure, thrombotic microangiopathy, hyper-reninaemia and hypertension. Her skin abnormalities are typical for scleroderma with sclerodactyly and acral ulceration (*figure 1*) and absence of wrinkling of her facial skin (*figure 2*).

Captopril 25 mg, three time a day was given. The blood pressure diminished to 140/90 mmHg and after adding labetolol to 120/70 mmHg. Prednisone was reduced to 5 mg/day and methotrexate was stopped. The platelet count and lactate dehydrogenase normalised. Renal function and plasma renin activity improved slowly. Symptoms of dyspnoea and acral ulcerations improved in the course of time. Two years after admission the patient was in a stable clinical condition with a creatinine of 146 µmol/l.

SRC is a syndrome that is characterised by accelerated hypertension, rapidly progressive renal failure, and hyper-reninaemia. Of note, hypertension blood pressure can be normal in SRC. The frequency of SRC in diffuse cutaneous systemic sclerosis (dcSSc) is 15 to 20%. The renin-angiotensin system plays an important role in its pathogenesis. Decreased blood flow, caused by structural vascular changes and possibly vasospasm (renal Raynaud's phenomenon),[1] lead to decreased renal perfusion. This causes excessive release of renin and subsequently formation of large amounts of angiotensin II, resulting in further vasoconstriction, raised blood pressure and renal ischaemia. The end result is a vicious cycle that is believed to be the cause of SRC.

Our patient had several characteristics indicating an increased risk for SRC: diffuse form of SSc, rapid progression of skin thickening and a duration of SSc less than four years.[2] Factors possibly provoking SRC in our patient were (1) daily intake of corticosteroids, (2) cold season and (3) cardiac dysfunction. (Ad 1) Corticosteroids increase ACE activity via inhibition of prostacyclin production and may thus contribute to the pathogenesis of SRC. Therefore, the dose of prednisone was reduced to 5 mg/day. (Ad 2) The patient developed SRC in the wintertime. SRC onset has been reported more often during winter, suggesting that renal Raynaud's phenomenon may be a contributing factor.[1] (Ad 3) The patient suffered from arrhythmias. Cardiac dysfunction precedes SRC in some patients.[2] All mentioned factors contribute to decreased renal perfusion, leading to excessive secretion of renin. The hyper-reninaemia maintains SRC via a vicious cycle (*figure 3*). The vicious cycle can be interrupted by ACE inhibitors. Since the introduction of ACE inhibitors one-year survival has increased from 10% to 76%.[2] ACE inhibitors inhibit the conversion of angiotensin I to angiotensin II, resulting in a prompt drop in blood pressure in many patients with SRC. In our patient, after treatment with captopril, the blood pressure decreased and renal function improved slowly.

Figure 3. *Pathophysiology of scleroderma renal crisis*



MAHA = microangiopathic haemolytic anaemia.

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MONTHLY NJM ONLINE HITLIST

The table lists online hits for all articles published in the June issue of the Netherlands Journal of Medicine, 2007 (available online on PubMed since 22 June 2007).

Article	Hits
EDITORIAL	
Power: not only a matter of numbers, but also of design	155
REVIEW	
Nocardiosis: a case series and a mini review of clinical and microbiological features	197
ORIGINAL ARTICLES	
The $Asp^{299}Gly$ Toll-like receptor 4 polymorphism in advanced aortic atherosclerosis	175
Risk calculation for hyperkalaemia in heart failure patients	153
CASE REPORTS	
Extremely high serum ferritin levels as diagnostic tool in adult-onset Still's disease	473
Life-threatening *Pneumocystis jiroveci* pneumonia following treatment of severe Cushing's syndrome	144
PHOTO QUIZZES	
Abdominal pain with unexpected pulmonary consequences	166
Neck swelling following a vigorous neck massage	220
LETTER TO EDITOR	
Fatal disseminated toxoplasmosis after liver transplantation: improved and early diagnosis by PCR	131
MONTHLY NJM ONLINE HITLIST	
For all articles published in March 2007	120
Total	**1934**

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

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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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The *Abstract*, not exceeding 250 words, should be written in a structured manner and with particular care. In original articles, the Abstract should consist of the following paragraphs: 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.

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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.

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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 funding sources should be credited here. Also a statement of conflicts of interest should be mentioned.

References should be numbered consecutively as they appear in the text (after the punctuation and in square brackets). Type the reference list with double spacing on a separate page. References should be in the language they are published in, conform the 'Vancouver' style for biomedical journals (N Engl J Med 1991;324:424-8).

Journal abbreviations should conform to the style used in the Cumulated Index Medicus. Examples:

1. Smilde TJ, van Wissen S, Wollersheim H, Kastelein JJP, Stalenhoef AFH. Genetic and metabolic factors predicting risk of cardiovascular disease in familial hypercholesterolemia. Neth J Med 2001;59:184-95.
2. Kaplan NM. Clinical Hypertension. 7th ed. Baltimore: Williams & Wilkins; 1998.
3. Powell LW, Isselbacher KJ. Hemochromatosis. In: Braunwald E, Fauci AS, Kasper DL, et al., editors. Harrison's Principles of Internal Medicine. 15th edition. New York: McGraw-Hill; 2001. p. 2257-61.

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Legends for figures should be typed, with double spacing, on a separate page.

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Case 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. Selection of case reports will be based on criteria as outlined in a special report by the editors (Drenth et al. The case for case reports in the Netherlands Journal of Medicine. Neth J Med 2006;64(7):262-4). We advise potential authors to take notice of the instructions in this report. Articles published in this section should be no longer than 1000 words, and 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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Mini reviews are concise notes that bring the reader up to date with the recent developments in the field under discussion. The review article should mention any previous important reviews in the field and contain a comprehensive discussion starting with the general background of the field. It should then go on to discuss the salient features of recent developments. The authors should avoid presenting material which has already been published in a previous review. The manuscript should be divided as follows: title page, abstract and main text. The text may be subdivided further according to the areas to be discussed. The text should not exceed 2500 words.

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Photo quiz
A photo quiz should not exceed 500 words and include no more than two figures and four references conform the Vancouver style. Abbreviations of measurements should be quoted in SI units.

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