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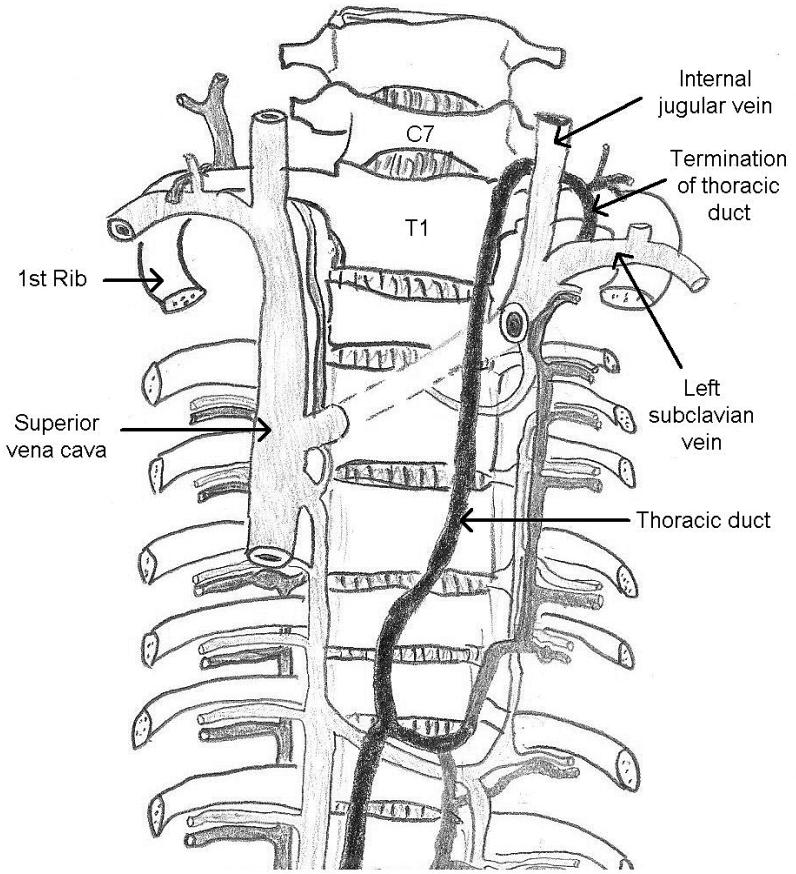

PHOTO QUIZ: The thoracic duct, see page 221

JUNE 2007, Vol. 65, No. 6, ISSN 0300-2977

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EDITORIAL

Power: not only a matter of numbers, but also of design

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In this issue, Hommels *et al.* reported a study on a possible relationship between the Asp[299]Gly polymorphism in the Toll-like receptor-4 (TLR-4) gene and advanced aortic atherosclerosis.[1] They did not find a significant relationship in their study, although others have reported a protective effect.[2,3] The authors comment that the conclusion of their study should be taken with caution because of the small number of subjects. The study raises the question whether small studies are useful to study the relationship between common polymorphisms and disease.

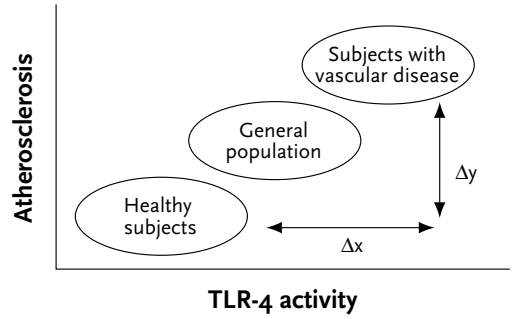
First of all, I would like to stress the enormous advantage of genetics in the search for causes of disease. Although the relationship between inflammation and cardiovascular disease has been known for many years, nongenetic observational epidemiological studies are hampered by confounding and reverse causality. For instance, if somebody does find a relationship between C-reactive protein (CRP) and cardiovascular disease in a case-control study, the question arises whether this relationship could be explained by an increase in CRP through cardiovascular disease or vice versa. Moreover, several other factors could explain such a relationship through confounding. Using the concept of Mendelian randomisation, Davey Smith and Ebrahim eloquently describe the advantages of the use of genetic determinants instead of plasma markers.[4] Besides this conceptual advantage, using genetic determinants also has some practical advantages. When DNA is isolated, many genetic determinants are easily available via high throughput facilities and genetic measurements are less influenced by storage conditions compared with the measurement of plasma markers. However, an important drawback of genetic studies is that in complex disease the effect of a single polymorphism is usually small and often dependent on the genetic and environmental background it is evaluated in. This requires not only an adequate sample size but also an adequate study design.

STUDY DESIGN

The study of the effect of a polymorphism on the incidence of a certain disease requires an effect measure which could be defined as the ratio between a difference in a certain determinant (X) and the difference in a certain outcome (Y). In case of the study on TLR-4 and atherosclerosis, one would like to know whether a change in TLR-4 activity is accompanied by a change in (incidence of) atherosclerosis (*figure 1*).

In fact, such a study could start with a contrast in X or with a contrast in Y. In other words one could start by recruiting a group of people that show a contrast in atherosclerosis and look for TLR-4 activity, or vice versa. Hommels *et al.* started their study with a group of patients with hypertension who underwent scanning of the abdominal aorta. The question is whether the contrast in atherosclerosis is comparable with a case-control study as carried out by Ameziane *et al.* who studied patients with vascular disease on the one hand and healthy subjects (hospital employees and blood donors) on the other.[3]

Figure 1. *The reliability of effect estimation of a possible relation between TLR-4 activity and atherosclerosis depends on the contrasts in X (TLR-4 activity) or in Y (atherosclerosis)*



The contrast in atherosclerosis may be smaller when all patients have hypertension, which is a risk factor for atherosclerosis. In fact the authors made a comparison between subjects with advanced aortic atherosclerosis and less advanced aortic atherosclerosis.

Figure 1 shows that a comparison between subjects with vascular disease and a healthy population shows the strongest contrast. However, if a certain effect is found one is not sure that it is an effect of the determinant on the occurrence of disease or on the occurrence of healthiness. Therefore, it is recommended to take the control group from the general population.

A second point is the contrast in X. The hypothesis is that a change in TLR-4 activity is accompanied by a change in atherosclerosis. Because TLR-4 activity is difficult to measure, the authors measured the $Asp^{299}Gly$ TLR-4 polymorphism, which is a genetic determinant of TLR-4 activity. This is a meaningful approach, which is in some ways better than measuring TLR 4 activity, because studies that use genetic determinants are less prone to confounding. However, only the wild-type and heterozygote genotypes were found. The question is whether there is enough contrast between wild-type and heterozygote genotype in TLR-4 activity.[5] If there is no difference in TLR-4 activity in wild-type and heterozygotes, the estimation of effect will strongly tend to no effect.

A third point is the question of confounding. Hommels *et al.* presented an uncorrected odds ratio. As stated before, studies that use polymorphisms as determinants are less prone to confounding than studies that use plasma markers. Therefore, the unadjusted estimate could be regarded as a good measure of effect. However, this does not rule out confounding. As shown in their table there are big differences in age. Therefore I would recommend also presenting adjusted odds ratios. This is comparable with the case of randomised trials with an uneven distribution of covariates. Randomisation should in theory result in an equal distribution of covariates, but if it does not this could allow confounding to occur. Another advantage of adjustment for age is that this would increase the contrast in atherosclerosis, which is strongly age dependent.

SAMPLE SIZE

The fourth point is on numbers. A genetic study of 123 subjects with a polymorphism that is supposed to give at the most a small effect is at least underpowered to find a significant effect. However, one should keep in mind that the point estimate of effect is not influenced by the sample size. Whether it is useful to publish the results of small studies is a matter of debate.

In a recent study Morgan et al. tried to validate the effects of multiple genes on atherosclerosis.[6] The authors screened the literature and found 96 polymorphic genetic variants in 75 genes that were positively associated with atherosclerosis. They subsequently screened 85 polymorphisms in 70 genes. Using appropriate statistical techniques, including correction for multiple testing, they did not find a positive correlation between any of the tested genes and the risk of atherosclerosis. This study nicely illustrates the drawbacks of this type of research.

A lot of small studies taken together make a big one. The question is whether such pooled analysis suffers from bias because journals tend not to publish 'negative' results. Recently, Borm *et al.* showed that publication bias is not a serious issue in meta-analysis of trials and it could be argued that the same holds for studies on genetic determinants of disease.[7] It could be argued that the same holds for studies on genetic determinants of disease, although it has been demonstrated that molecular genetic research is more sensitive to publication bias than clinical trials.[8]

In conclusion, I don't argue that we should stop publishing small studies, but it is important to be very reserved in the conclusions based on such small studies, whether 'negative' or 'positive'- as is done in the paper of Hommels et al. Firm conclusions should be based on multiple, large-scale studies.

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Den Heijer. Power: not only a matter of numbers, but also of design.

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REVIEW

Nocardiosis: a case series and a mini review of clinical and microbiological features

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ABSTRACT

Infections caused by *Nocardia* species are uncommon and have a wide variety of clinical manifestations in immunocompetent and immunocompromised patients. The diagnosis of nocardiosis can easily be missed because there are no characteristic symptoms.

We present one case of a *Nocardia* infection in detail and give a brief description of eight other cases, including a relatively unique type of *Nocardia veterana*, diagnosed in our hospital during a five-year period. The diversity of clinical manifestations, microbiological identification and general principles of treatment of nocardiosis are reviewed.

KEYWORDS

Immunocompromised, *Nocardia, Nocardia veterana,* nocardiosis, sulphonamide, trimethoprim

INTRODUCTION

Nocardiosis is an uncommon bacterial infection with a wide variety of clinical manifestations in immunocompetent and immunocompromised patients. The incidence of nocardiosis in the United States has been estimated to be 500 to 1000 cases per year.[1] The number of cases reported in the literature is increasing. This might be due to an absolute increase in the number of immunocompromised patients but also to improvement in laboratory techniques to detect nocardiosis. Most patients with nocardiosis have an immune dysfunction due to haematological malignancies, other malignancies, organ transplantation, AIDS, liver cirrhosis, diabetes, alcoholism or corticosteroid use.

CASE REPORT

A 76-year-old man presented with painful upper legs, proximal muscle weakness, morning stiffness in the pelvic girdle and headache. On suspicion of polymyalgia rheumatica or giant-cell arteritis (biopsy of the temporal artery was negative), he was treated with 60 mg prednisolone daily, without any effect.

Three months later, he was admitted because of severe pain in the left upper leg for one week. There was no trauma or fever. Physical examination revealed erythema at the medial side of the left upper leg (10 x 20 cm). Laboratory investigation showed an increase in the erythrocyte sedimentation rate (ESR, 38 mm/h), high C-reactive protein level (CRP, 116 mg/l) and leucocytosis (15.9 x 10^9/l). The serum creatinine kinase was normal; lactate dehydrokinase (LDH) was slightly elevated (593 U/l). Ultrasound investigations indicated an elongated collection of fluid at the medial side of the upper leg (4.9 cm x 10 cm). A computerised tomography (CT) showed an abscess in the adductor compartment with indurations of the gracilis muscle and the subcutaneous fat. The abscess was evacuated surgically. In the gram stain, branching gram-positive rods were observed. The culture yielded *Nocardia farcinica*.

Treatment with trimethoprim-sulphamethoxazole (TMP-SMX) 1920 mg twice daily for three months was successful. After three weeks, the CRP was <5 mg/l with normal leucocyte counts. The ESR was still slightly elevated (31 mm/h).

Eighteen months after cessation of the antibiotics, the patient developed renal insufficiency, due to a Wegener's granulomatosis. He was treated successfully with high-dose prednisolone and cyclophosphamide. There were no signs of reactivation of the *Nocardia* infection.

DISCUSSION

During the period from January 2000 to July 2004 nine cases of nocardiosis were diagnosed in our general 600-bed teaching hospital with facilities for autologous stem cell transplantation, rheumatic diseases and follow-up after lung transplantation (*table 1*).

Most patients were infected with *Nocardia farcinica* or *Nocardia asteroides* complex. We found one case of *Nocardia veterana* (case 5). Little is known about *Nocardia veterana* because it is infrequently reported as a pathogen.[2-7] It is named after the veterans hospital, where it was first isolated. The first report of *N. veterana* isolated from human samples was by Gurtler in 2001.[2] Pulmonary nocardiosis was observed in seven patients, primary cutaneous nocardiosis in one patient and systemic nocardiosis in one patient. The time to diagnosis varied from two to seven days.

Eight patients could be considered to be immunocompromised as part of their underlying disease or treatment. Most patients (cases 1, 2, 4, 6 and 8) were treated with various kinds of chemotherapy and immunotherapy, because of cancer. Three patients were on immunosuppressive medication because of lung transplantation (case 5), polymyalgia rheumatica (case 3) or COPD (case 9). Most patients were treated with TMP-SMX. The duration of therapy varied from 1 to 630 days. One patient died within one day and one patient was not treated. Of the remaining seven patients, six patients recovered. One patient was treated for two years, the other four patients for 14 to 90 days.

DIAGNOSIS

Nocardia species are found worldwide in soil, decaying vegetable matter and aquatic environments.[8] The main route of acquisition is through direct inhalation of contaminated particles or by direct inoculation through the skin. The manifestations of nocardiosis can be solely pulmonary, but *Nocardia* species can also disseminate from a pulmonary or cutaneous focus to virtually any organ. The onset of pulmonary nocardiosis may be acute, subacute or chronic. Due to its nontypical manifestations nocardiosis is frequently misdiagnosed.

The initial diagnoses are pneumonia, tuberculosis and carcinoma or lung abscesses. Fatigue, fever, chills, productive cough, dyspnoea, pleural chest pain and loss of weight can occur in patients with nocardiosis. Cutaneous nocardiosis leads to cellulitis, pustules, pyoderma, paronychia, ulcerations or localised abscesses.

Table 1. *Characteristics of nine Nocardia infections*

Case	Species	Localisation	Time to diagnosis (days)	Disease	Antibiotic treatment	Duration of therapy (days)	Outcome
1	*Nocardia farcinica*	Disseminated: lung, gluteal region, iliac fossa, kidney, cerebrum	6	NHL	TMP-SMX and imipenem, followed by amikacin and TMP-SMX, followed by TMP-SMX	630	Recovered
2	*Nocardia farcinica*	Lung	6	MM	TMP-SMX 2 x 1920 mg iv	60	Recovered
3	*Nocardia farcinica*	Upper leg abscess	2	PMR	TMP-SMX 2 x 1920 mg iv	90	Recovered
4	*Nocardia asteroides* complex	Lung	4	CLL	TMP-SMX 2 x 1920 mg iv, followed by amikacin 2 x 375 mg iv and meropenem 3 x 1 g iv	20	Death
5	*Nocardia veterana*	Lung	1	LTX, because of interstitial fibrotic lung disease	TMP-SMX 3 x 1920 mg iv, followed by imipenem and amikacin iv followed by minocycline orally	30	Recovered
6	*Nocardia asteroides* complex	Pneumo-nectomy cave	2	Metastatic NSCLC, chemotherapy	TMP-SMX 2 x 960 mg iv	14	Recovered
7	*Nocardia asteroides* complex	Lung	7	Bronchogenic cyst	Ciprofloxacin 2 x 500 mg	60	Recovered
8	*Nocardia* spp.	Lung	2	Metastatic prostate carcinoma	Imipenem 4 x 500 mg, amikacin 15 mg/kg iv	1	Death
9	*Nocardia* spp.	Lung	-	Bronchiectasis	No treatment	-	Death due to COPD and heart failure

NHL = non-Hodgkin lymphoma; TMP-SMX = trimethoprim-sulphamethoxazole; MM = multiple myeloma; PMR = polymyalgia rheumatica; CLL = chronic lymphocytic leukaemia; LTX = lung transplantation; NSCLC = non-small-cell lung carcinoma; COPD = chronic obstructive pulmonary disease.

Figure 1. *CT scan shows an abscess in the left iliac fossa due to disseminated nocardiosis*

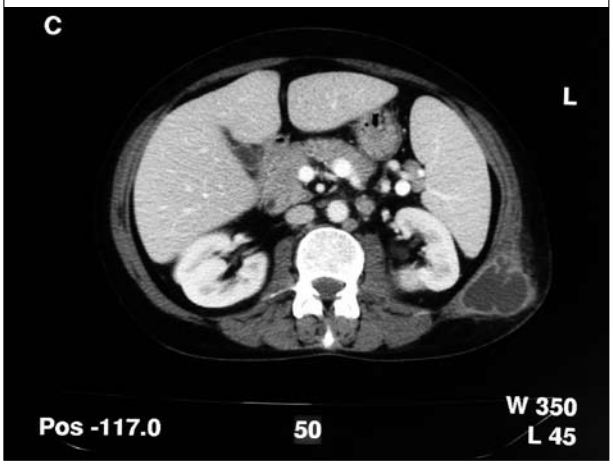


Figure 2. *CT scan reveals pulmonary infiltrates in a patient with disseminated nocardiosis*

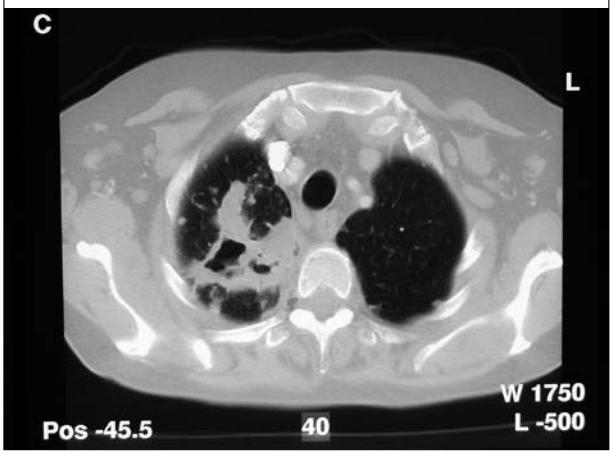
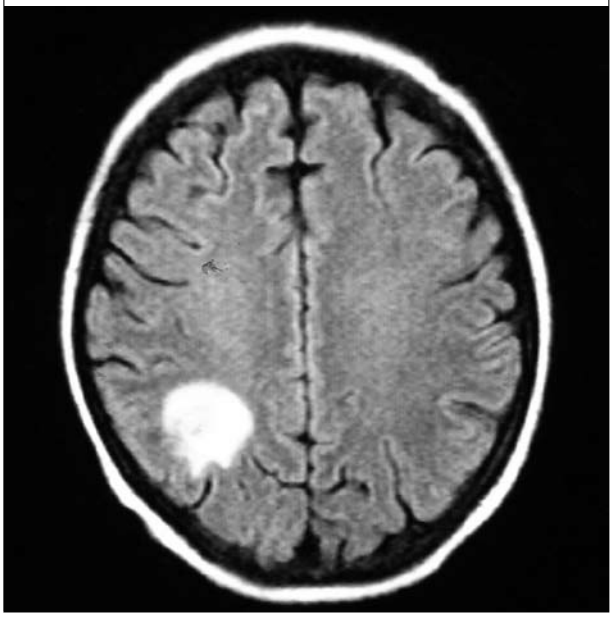


Figure 3. *Magnetic resonance imaging shows signs of an abscess in the right occipital cerebrum*



Haematogenous spread most commonly involves the central nervous system, bone, retina, heart, joints and kidneys. The presence of lesions in two or more organs of the body defines systemic or disseminated disease.[9-11]

The genus of *Nocardia* is rapidly expanding and consists of at least 22 species. The most frequently isolated species belong to the *N. asteroides* complex, which is a heterogeneous group that includes *N. asteroides* sensu strictu, *N. farcinica, N. nova* and *N. abscessus.*[12] Other medically important species are *N. brasiliensis, N. otitidis-caviarum, N. africana, N. brevicatena* complex, *N. carnea, N. paucivorans, N. pseudobrasiliensis, N. transvalensis* and *N. veterana.* Identification of clinical isolates beyond the genus level is important since *Nocardia* species differ in the clinical spectrum of the disease they cause and their susceptibility to antibiotics. In particular, *N. farcinica* is much more resistant than other *Nocardia* species.[13]

The genus of *Nocardia* belongs to the family of aerobic actinomycetes characterised as gram-positive branching filamentous rods producing fungus-like colonies in culture.[12] *Nocardia* species can be recovered on isolation media for bacteria, fungi or mycobacteria, but growth is slow and incubation should be continued for at least two weeks. They can grow at high temperatures (37° to 45°C) and growth is accelerated by CO_2. Premature discontinuation of culture will decrease the sensitivity of recovery and may contribute to underestimation of the true incidence of nocardiosis. Typically, colonies are chalky white, but they can also be yellow, pink or orange. A characteristic smell is produced, vividly described as a musty basement odour or earthy smell. Further characteristics that help to identify *Nocardia* in the laboratory are its partial acid-fastness, lysozyme resistance and hydrolysis of casein, tyrosine, xanthine and hypoxanthine. After presumptive identification, final determination is nowadays accomplished in reference labs by molecular techniques, such as 16S rRNA sequence analysis, restriction fragment length polymorphism (RFLP) or polymerase chain reaction (PCR).

TREATMENT

Management of nocardiosis involves antimicrobial therapy in conjunction, where appropriate, with surgical debridement/drainage and improvement of immune function if feasible. Therefore, the choice of therapy depends on the severity and localisation of the infection, the host immune status, potential drug interactions and toxicity and the *Nocardia* species involved. In general, nocardiosis has been treated successfully with sulphonamides since the early years of antimicrobial therapy.[14,15] Combined with TMP synergy occurs, and TMP-SMX has been the mainstay treatment of *Nocardia* infections. Probably due

to the low prevalence of nocardiosis, studies prospectively comparing TMP-SMX with sulphonamide monotherapy or other antibiotic regimens have not been performed. Duration of therapy has not been systematically evaluated either, but it is generally advised to treat cutaneous forms of *Nocardia* infection for one to three months, patients with pulmonary or systemic nocardiosis for six months and those with involvement of the central nervous system for 12 months.[13,15] All immunocompromised patients should be treated for at least one year.[13,15] In the present series, we observed resolution in six of nine patients. In five patients the therapy was three months or less. In some cases it might be possible to give shorter treatment than recommended in the literature. For adults with normal renal function, the recommended daily dose is 5 to 10 mg/kg TMP and 25 to 50 mg/kg SMX, divided over two to four doses. For the treatment of cerebral abscesses, severe disseminated disease and AIDS patients, a higher initial daily dose can be considered: 15 mg/kg TMP and 75 mg/kg SMX.[15] After three to six weeks, dosage can generally be reduced and treatment can be continued orally.

In general, TMP-SMX is well tolerated, but side effects may occur. These include gastrointestinal symptoms, skin disorders, renal impairment, hepatotoxicity and bone marrow failure.[16] During the prolonged treatment regimens required for nocardiosis, side effects occur more frequently, especially in AIDS patients. In these patients, a high incidence of rash, fever and neutropenia has been noted.[17,18] In addition, 20% of AIDS patients develop hepatotoxicity upon treatment with TMP-SMX.[17] One of the most serious side effects is bone marrow suppression related to folate deficiency. Patients may develop pancytopenia, or more rarely and less well understood agranulocytosis, anaemia or thrombocytopenia.[18] Supplementation of folinic acid could prevent folate depletion and the related side effects. Serious side effects such as neutropenia may necessitate a switch to alternative antimicrobial agents, belonging to the carbapenems, cephalosporins, aminoglycosides, quinolones, macrolides or tetracyclines.

The use of TMP-SMX may be further complicated by the occurrence of resistance. Reports on resistance are sporadic and hampered by the fact that no universally accepted antimicrobial susceptibility testing method has been established for *Nocardia* species.[14] In general, *Nocardia* species are still considered susceptible to most antimicrobial agents, except *N. farcinica* and *N. otitidiscaviarum*. *N. farcinica* is resistant towards ampicillin, third-generation cephalosporins, erythromycin, gentamicin and tobramycin.[13] *N. farcinica* remains susceptible to amikacin, imipenem, ciprofloxacin and TMP-SMX. The clinical outcome of therapy depends on the site of infection, the comorbidity and underlying host factors. Mortality is high among immunocompromised patients and those with multiple brain abscesses (75 to 90%). Disseminated nocardiosis has a poor prognosis with a mortality rate >85% in immunocompromised hosts.[9,13,14]

CONCLUSION

Due to its low incidence and nontypical manifestations, nocardiosis is frequently misdiagnosed while recognition of this infection is important for the choice of appropriate antibiotic treatment. Treatment duration has to be at least months. Despite prolonged treatment, mortality remains high, especially in immunocompromised patients.

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ORIGINAL ARTICLE

The Asp^{299}Gly Toll-like receptor 4 polymorphism in advanced aortic atherosclerosis

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ABSTRACT

Background: Recently, the common Asp^{299}Gly polymorphism of the Toll-like receptor 4 (TLR-4) was found to be associated with a reduced incidence of acute myocardial infarction and carotid atherosclerosis. As TLR-4 signalling is causally involved in atherogenesis, the polymorphism was postulated to impart protection from atherosclerosis. To explore a potential atheroprotective effect, we studied the association between the Asp^{299}Gly polymorphism and atherosclerosis in hypertensive patients undergoing angiography for suspected renovascular disease.

Methods: 140 hypertensive subjects underwent intra-arterial digital subtraction angiography, during which the presence of atherosclerotic lesions was assessed at the level of the abdominal aorta and renal arteries. Extensiveness of disease was classified as follows: atherosclerosis confined to the abdominal aorta, unilateral renal artery stenosis or bilateral renal artery stenosis. Subsequently, genotyping for the +896 A>G (Asp^{299}Gly) single nucleotide polymorphism was performed in all patients. In statistical analyses 17 patients were excluded because of incomplete data (n=3) or a diagnosis of fibromuscular disease (n=14).

Results: 21 patients were found heterozygous for the ^{299}Gly allele, whereas none of the subjects were ^{299}Gly homozygous (^{299}Gly allele frequency 7.8%). The prevalence of the ^{299}Gly allele in atherosclerotic patients was not different from the prevalence observed in subjects without atherosclerotic lesions (16.9 *vs* 15.5%, p=0.83). Moreover, ^{299}Gly carriership was not associated with the extensiveness of (advanced) aortic atherosclerosis (p=0.64).

Conclusion: Our results suggest that the Asp^{299}Gly TLR-4 receptor polymorphism is not associated with the prevalence nor extensiveness of (advanced) aortic atherosclerosis.

KEYWORDS

Angiography, atherosclerosis, hypertension, Toll-like receptor

INTRODUCTION

Mounting evidence suggests that specific (infectious) agents enhance arterial inflammation during atherogenesis, based on their interaction with receptor signalling pathways of the innate immune system.[1,2] Consequently, Toll-like receptor 4 (TLR-4) induced signalling has been described in chronic low-grade arterial inflammation.[3]

TLR-4 is well known as a pattern-recognition receptor for exogenous lipopolysaccharide (LPS) derived from gram-negative bacterial infection.[4,5] Although several other ligands such as fibrinogen,[6] fibronectin,[7] heat-shock protein,[8] hyaluronan oligosaccharide[9] and minimally modified low-density lipoproteins (LDL)[10,11] have also been described, the exact nature of TLR-4 engaged signalling in atherosclerosis remains elusive.

Although rather speculative, several authors[12,13] have postulated that the advantages of a prominent TLR-4 mediated inflammatory response and subsequent containment of pathogens are outweighed by the unremitted receptor response to endogenously derived epitopes (e.g. oxidised LDL) during atherogenesis. Thus, an attenuated TLR-4 response might confer a potential advantage, as progression of atherosclerosis will decline.

In this context, recent clinical research has described a common Asp^{299}Gly TLR-4 receptor polymorphism associated with a blunted receptor activity and a subsequently diminished inflammatory response.[2,5,14] According to an Asp^{299}Gly based attenuated receptor signalling and a subsequently hypothesised reduced atherogenesis, ultrasound analysis of carotid arteries in the

Bruneck study[14] showed that the Asp[299]Gly polymorphism was found less frequently in patients with progressive carotid lesions, when compared with a control group.

Although a potential Asp[299]Gly mediated protective cardiovascular effect has since been studied extensively, clinical research has focused on acute coronary events,[12,15-22] while data regarding Asp[299]Gly prevalence in peripheral atherosclerosis have remained remarkably scarce.[14,23-25] (See *figure 1* for an overview of published case-control studies).

Yet critical appraisal of clinical reports merely demonstrates a consistent trend towards a reduced frequency of the Asp[299]Gly TLR-4 polymorphism in patients with acute myocardial infarction,[15,18,20] whereas progression of coronary stenosis was found unaffected by genetic TLR-4 variants.[12,21] Moreover, a protective effect based on [299]Gly carriership in early atherosclerosis remained inconclusive.[14,23,24]

Therefore, the present study was conducted to explore the association between the Asp[299]Gly polymorphism and atherosclerosis in hypertensive patients undergoing angiography for suspected renovascular disease. Since renovascular disease is generally considered to be advanced systemic atherosclerosis,[26] we hypothesised a higher frequency of the Asp[299]Gly TLR-4 polymorphism in patients without angiographically demonstrated atherosclerotic lesions in the abdominal aorta and/or renal artery.

MATERIALS AND METHODS

Subjects

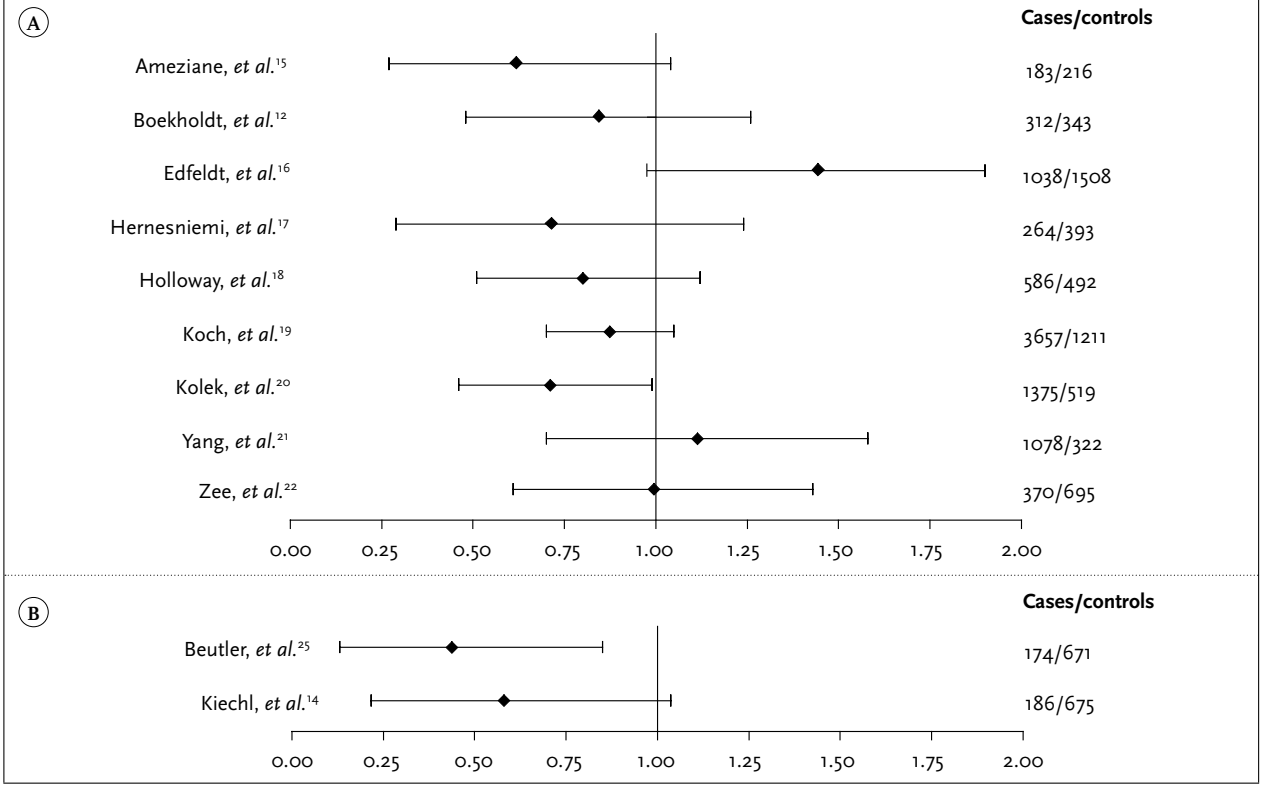
All 140 hypertensive patients included in the present study underwent angiography when one or more of following criteria were present: treatment-resistant hypertension (elevated blood pressure despite ≥3 adequately dosed antihypertensive drugs), >20% increase in serum creatinine concentrations induced by an angiotensin-converting enzyme inhibitor, smoking and diastolic blood pressure >110 mmHg, malignant or accelerated hypertension, extra-renal atherosclerosis in ≥2 different vascular beds.[27-29]

Other causes of secondary hypertension were excluded biochemically before patients underwent angiography of the renal arteries. Written informed consent was obtained from all patients and the Medical Ethical Committee of Maastricht University Hospital approved the study protocol.

Angiography

The angiographic procedure was carried out in the angiosuite of the Department of Radiology in Maastricht University Hospital. Intra-arterial digital subtraction angiography (DSA) was performed with a commercially available digital subtraction system (Integris 5000; Philips Medical Systems;

Figure 1. *Relative risk of acute coronary events (A) and extracoronary atherosclerosis (B) (crude odds ratio and 95% confidence interval) in carriers of the [299]Gly TLR-4 variant allele*



Best, the Netherlands). Angiographic images of the abdominal aorta and renal arteries were obtained in anteroposterior, and left and right oblique views with injection of 30 ml iohexol (Omnipaque 300; Nycomed, Oslo, Norway) through a 4-F Universal Flush catheter (Cordis Europe, Roden, the Netherlands) positioned at the level of the renal arteries.

Radiological evaluation

DSA images were reviewed by two independent radiologists for the presence or absence of atherosclerotic lesions in both renal arteries and the abdominal aorta (celiac truncus up to iliac bifurcation). Subsequently, extensiveness of atherosclerosis was scored as either lesions confined to the abdominal aorta, unilateral renal artery stenosis or bilateral renal artery stenosis. Final results were based on consensus. Patients in whom fibromuscular dysplasia had been diagnosed were excluded from analysis.

Laboratory measurements

Blood samples were drawn after an overnight fast. Plasma cholesterol and glucose were determined using standard methods with commercially available kits. Serum creatinine was measured on the Beckman Coulter Synchron LX-20 system (Beckman Coulter, Inc Fullenton, CA, USA). Creatinine clearance was calculated using the Cockcroft and Gault formula.[30]

Moreover, peripheral blood cells were obtained by standard procedures involving ultracentrifugion and the cell fraction obtained was stored on a phosphate buffered saline or a nucleic acid sequence-based amplification buffer (QIagen, CA, USA) at - 80°C until analysis.

DNA isolation and polymerase chain reaction

DNA isolation from peripheral blood cells was performed using the WIZARD method (Promega, CA, USA), according to the manufacturer's instructions. Amplification was performed in 40 cycles, starting at 94°C for four minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C (30 seconds) and extension at 72°C (30 seconds). The polymerase chain reaction (PCR) was performed in a reaction mixture containing 2 µl $MgCl_2$, 1 µl dNTPs, 0.25 µl Taq, 1 µl forward primer and 1 µl reverse primer (New England Biolabs, MA, USA).

PCR fragments were digested using the NCO_1 enzyme (New England Biolabs, MA, USA) and the digested products were tested on a 2.5% agarose gel stained with ethiumbromide. Restriction fragments were visualised using the Bio-Rad Multi-Analyst ™/PC version 1.1 (BioRad, CA, USA). Two researchers independently scored the genotype in a blinded fashion.

Statistical analysis

All data are represented either as mean and standard deviation, or median and ranges in case of nonparametric distribution. In case of normally distributed data, differences were assessed using a two-sided t-test and Mann-Whitney testing was applied in case of deviation. Dichotomous data were compared using χ^2 statistics. The Hardy-Weinberg equilibrium was tested using standard methods.[31]

To assess the association between TLR-4 genotype and advanced atherosclerosis, subjects were stratified based on the presence/absence of angiographically demonstrated lesions in either aorta or renal arteries. Subsequently, a distinction was made based on the presence of unilateral or bilateral renal artery stenosis (extensiveness of atherosclerosis). To explore potential interactions between Asp^{299}Gly genotype and atherosclerosis logistic regression analysis was adapted.

A two-sided p value <0.05 was considered statistically significant. Analyses were performed with SPSS software (SSPS version 11.0, IL, USA).

RESULTS

Among 140 hypertensive subjects genotyped, 21 patients were heterozygous for the Asp^{299}Gly TLR-4 allele. None of the subjects were ^{299}Gly homozygous. Subsequently, an overall Asp^{299}Gly allele frequency of 7.8% was calculated. Allele frequencies did not deviate from the Hardy-Weinberg expectations (p=0.64).

To determine whether the presence of the Asp^{299}Gly polymorphism decreased susceptibility to and extensiveness of atherosclerotic disease, subsequent statistical analyses were based on 123 patients. Seventeen patients were excluded from analysis because of missing data (n=3) or a diagnosis of fibromuscular dysplasia (n=14). Clinical characteristics of all patients analysed are presented in *table 1*.

Angiographic imaging revealed atherosclerotic lesions in 65 patients (52.8%). In 24 cases lesions were confined to the abdominal aorta, while most patients (n=30) displayed lesions of both aorta and renal artery. In 25 patients bilateral renal artery stenosis as part of advanced atherosclerosis was diagnosed.

When patients were stratified according to the presence of atherosclerotic lesions in either aorta or renal arteries, a 16.9% prevalence of the Asp^{299}Gly mutation in atherosclerotic subjects *vs* 15.5% in subjects without atherosclerotic lesions was found (p=0.83, *table 2*). Moreover, an association between Asp^{299}Gly carriership and the extensiveness of advanced atherosclerosis appeared to be lacking (R=0.89, p=0.64).

Although distribution of several cardiovascular risk factors differed significantly between patients with and those without atherosclerotic lesions (*table 1*), none of these factors showed an interaction with both atherosclerosis and the Asp^{299}Gly genotype.

Table 1. *Patients characteristics*

	All patients (n=123)	Atherosclerosis (n=65)	No atherosclerosis (n=58)	P value
Age (years)	55.0±13	61±11	48±12	0.000*
Gender (male/female)	82/41	52/13	30/28	0.001*
Body mass index (kg/m^2)	27.6±5.0	26.7±5.9	28.7±3.9	0.037*
Current smokers (%)	36.6	43.1	29.3	0.130
Diabetes (%)	9.8	14.1	5.1	0.100
Cholesterol (mmol/l)	5.7	5.5	5.7	0.156
Range	2.6-8.7	2.6-8.7	3.5-8.7	
Glucose (mmol/l)	5.9	6.0	5.6	0.015*
Range	3.4-14.7	3.4-14.7	4.1-10.0	
Serum creatinine (µmol/l)	102.0	116.0	90.5	0.000*
Range	51-404	64-404	51-173	
Creatinine clearance (ml/min)	81.1±33.7	66.7±25.2	97.3±34.9	0.000*

* **Difference is statistically significant.**

Table 2. *Genotype and allele frequencies of the Asp299Gly polymorphism in subjects with and without documented atherosclerosis*

Genotypes	Atherosclerosis n (%)	No atherosclerosis n (%)	Odds ratio	95% CI
Asp/Asp	54 (83.9)	49 (84.4)		
Asp/Gly	11 (16.9)	9 (15.5)	1.1	0.42-2.90
Gly/Gly	0 (0)	0 (0)		
Frequency of the Gly allele	0.085	0.078		

Post hoc analysis showed that exclusion of patients with fibromuscular dysplasia did not influence the main results of this study.

DISCUSSION

Based on the assumption of an attenuated Asp^{299}Gly receptor function and a consequently blunted inflammatory response in atherogenesis, we expected carriers of the ^{299}Gly allele to be less prone to develop atherosclerosis. In contrast to our hypothesis and in spite of the intriguing findings described in previous reports,[14,15,17,18,20,25] the present study obtained no association between the Asp^{299}Gly TLR-4 receptor polymorphism and advanced aortic atherosclerosis.

Although an explanation for these negative findings is not readily apparent, our results might reflect the absence of Asp^{299}Gly homozygosity in our study. In this context, *in vitro* research[1,4,5,32-35] has obtained contradictory results regarding the functional relevance of a heterozygous Asp^{299}Gly TLR-4 mutation, whereas a functional association between impaired receptor function and Asp^{299}Gly homozygosity is rather well established.[1,4,5,35] In keeping with these findings, some clinical studies have described an impaired inflammatory response,[20,36] a lower incidence of coronary plaques[15,17,20] and a reduced progression of carotid atherosclerosis[36] in heterozygous carriers of the ^{299}Gly allele, while other reports could not corroborate these observations.[16,21,22] Given that the present study, like most other associated studies,[12,15,17,18,21] hones in on an attenuated receptor function without exploring *in vivo* cytokine production, the above alluded discrepancies may thus have consequences for the interpretation of our data.

However, considering the complex nature of atherosclerosis and the fact that the totality of the reported data does not unequivocally demonstrate a decreased atherosclerotic burden in carriers of the Asp^{299}Gly polymorphism (*figure 1*), it seems conceivable that Asp^{299}Gly carriership has only a minor impact in atherosclerosis. As a corollary, large epidemiological studies are required in order to address the impact of the Asp^{299}Gly polymorphism in atherosclerosis and hence the setting for genetic association studies, such as the present one, is rapidly disappearing.

Although all patients recruited in the present study underwent angiography based on clinical criteria creating a population with a rather constant prevalence of atherosclerosis, we recognise that there are potential drawbacks to our study which might have yielded false-negative results.

Most conspicuous is the rather small study size and a consequently restricted statistical power to detect minor differences. Exclusion of patients with fibromuscular dysplasia made our study even smaller, but it was imperative in order to reduce potential bias due to the inevitable misclassification of these subjects as not having atherosclerosis. Moreover, exclusion did not affect the main results of this study.

Another caveat might be the recruitment of high-risk patients as controls. Despite the careful characterisation of both cases and controls, a potential prevalence of subclinical atherosclerosis outside the angiographically assessed vasculature cannot be excluded. However, given the fact that we did not observe an association between

the occurrence of evident cardiovascular risk factors and Asp[299]Gly heterozygosity, we expect that a potential distortion due to subclinical atherosclerosis is less likely.

In conclusion, our results suggest that Asp[299]Gly heterozygosity has no effect on the prevalence nor extensiveness of advanced aortic atherosclerosis.

NOTE

Part of this work was presented at the European Society of Hypertension meeting in Madrid in June 2006.

ACKNOWLEDGMENTS

The authors wish to acknowledge Gerly van Vleuten, Anneke Heijmans and Karin Flobbe for their excellent technical assistance and advice.

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Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Risk calculation for hyperkalaemia in heart failure patients

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ABSTRACT

Background: We aimed to develop a model to estimate the risk of hyperkalaemia in patients treated for heart failure in a tertiary reference hospital and to identify precipitating factors.

Methods: 125 congestive heart failure (CHF) patients were studied retrospectively. Thirty of these patients developed episodes of hyperkalaemia (K ≥5.5 mmol/l). Both groups were compared for possible risk factors for hyperkalaemia (age, glomerular filtration rate (GFR), New York Heart Association (NYHA) class, diabetes mellitus (DM), ejection fraction and medication use (ACE inhibitors, angiotensin receptor blockers, aldosterone antagonists).

Results: On multivariate logistic regression analysis DM (OR 2.9, 95% CI = 1.05 to 8.3, p=0.041), GFR <45 ml/min (OR 4.1, 95% CI = 1.6 to 10.5, p=0.004) and NYHA class III-IV (OR 2.4, 95% CI = 0.9 to 6.3, p=0.086) were independently associated with hyperkalaemia, whereas age, ejection fraction and medication sort and dose were not. Of the episodes of hyperkalaemia, 38% were precipitated by periods of dehydration (diarrhoea, fever) or change of medication.

Conclusion: We identified kidney function, diabetes mellitus and heart failure class as independent risk factors of hyperkalaemia. The majority of the hyperkalaemic episodes develop without a precipitating factor. This implies that heart failure patients in a tertiary reference hospital should be very closely monitored to minimise the risk for hyperkalaemia.

KEYWORDS

Diabetes mellitus, heart failure, hyperkalaemia, renal failure, risk factors

INTRODUCTION

Adverse drug reactions are a major cause of hospitalisation. Recently it was reported that 6% of all hospitalisations are due to adverse drug reactions. Despite the fact that angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are viewed as safe drugs, they can be found in the top five of drugs leading to hospitalisation because of adverse drug reactions, leading to renal dysfunction and electrolyte disturbances.[1]

In 1999 it was found that adding spironolactone to ACE inhibitors reduced morbidity and mortality in patients with congestive heart failure (CHF)[2] and since than this combination has been used more and more. The combined use of ACE inhibitors and spironolactone, however, increases the risk of hyperkalaemia, as was found a few years after this policy had been generally accepted.[3-6] This was partly due to the fact that also patients with severe kidney failure (who were excluded from the RALES study) were treated with this combination. In addition advanced age, diabetes mellitus (DM), volume depletion, severity of chronic heart failure (NYHA) and use of nonsteriodal anti-inflammatory drugs (NSAIDs) are possible risk factors for hyperkalaemia in CHF patients on combined ACE inhibitors and aldosterone antagonists. Since all of these risk factors are linked to diminished kidney function, renal dysfunction appears to be a pivotal factor for this risk. Especially in older patients, renal dysfunction is often underestimated, since in these patient creatinine tends to be lower due to loss of muscle mass.

The purpose of this study is to provide the clinician with tools to identify patients at risk for hyperkalaemia in an outpatient setting so that he can take measures to prevent it or decide to monitor these patients more closely. To this end we aim to identify and quantify risk factors for hyperkalaemia. Secondly we aim to identify precipitating factors that are responsible for hyperkalaemic episodes.

METHODS

Study population

This is a retrospective study performed at the heart failure outpatient clinic of the Radboud University Medical Centre (a tertiary reference hospital). Only patients treated for at least three months between January 2002 and April 2006 were included. From these, a random sample of 128 patients was taken. Patients were excluded if they were under eighteen years (no patients) or on haemodialysis (three patients). This yielded a study group of 125 patients. In all patients, the diagnosis of heart failure had been made in the past.

Procedures

Medical records were reviewed, and the following parameters were collected: age, gender, NYHA class, left ventricular ejection fraction (LVEF), left ventricular end-diastolic diameter (LVEDD), a diagnosis of diabetes mellitus (DM), medication use and dose (ACE inhibitors, ARBs, aldosterone antagonists, β-blockers and diuretics) and laboratory variables (serum potassium and creatinine). Creatinine clearance was calculated using the Cockcroft-Gault formula.[7] In order to be able to compare different drugs within a class the prescribed daily dose was divided by the defined daily dose (ddd) of that class. In addition, it was assessed whether episodes of transient deterioration in kidney function occurred during the study period. Transient renal failure was defined as a 25% increase and subsequent decrease in serum creatinine within a period of three months, with a creatinine peak >110 μmol/l. Next patients were identified who had a potassium of ≥5.5 μmol/l during the study period. This 'case' group was compared with a control group that had no hyperkalaemia during the study period. For this latter group the most recent data available in the medical records were used for the subsequent analysis. For the case group the most recent episode of hyperkalaemia was taken and data were collected at three months, both prior to this episode and during the episode itself. An episode of hyperkalaemia was defined as any episode of elevated potassium (K ≥5.5 μmol/l). High potassium values occurring within a period of two weeks were considered to belong to the same episode of hyperkalaemia. In this way it was possible to both assess risk factors that lead to a hyperkalaemic episode and which precipitating factors directly provoke hyperkalaemia. Since we were interested in out-of-hospital risk factors of hyperkalaemia, episodes that were the consequence of renal failure due to radiocontrast were not included in the analysis.

Statistical analysis

A desktop computer equipped with SPSS 12.0.1 for windows was used for data analysis. Fisher's exact test and independent t-test were used for dichotomous and continuous variables respectively, comparing cases and controls on each of the collected variables. Variables found to have a p value <0.05 were incorporated into the logistic regression model. Variables independently associated with hyperkalaemia were identified. Based on the multivariate analysis a model was developed to predict the risk of hyperkalaemia in individual patients.

RESULTS

Thirty patients had 52 episodes of hyperkalaemia; 19 patients had one episode of hyperkalaemia, five patients had two episodes, four patients had three episodes, one patient had four episodes and one patient had seven episodes. In *table 1* data on the study population and findings in the case and control group can be found.

On multivariate logistic regression analysis DM (OR 2.9, 95% CI = 1.05 to 8.3, p=0.041), GFR <45 ml/min (OR 4.1, 95% CI = 1.6 to 10.5, p=0.004) and NYHA class III or IV (OR 2.4, 95% CI = 0.9 to 6.3, p=0.086) were independently associated with hyperkalaemia. Age, ejection fraction and medication sort and dose, were not independently associated with hyperkalaemia. All of the patients were taking at least one potassium-increasing drug (ACE inhibitor, ARB or aldosterone antagonist). None of them were treated by all three drug classes. There was no association between the number of potassium-modulating drugs and the occurrence of hyperkalaemia. Based on these ORs a model was designed, assigning 1 point to DM, 1 point to NYHA class III or IV and 2 points to GFR <45 ml/min. In *table 2* a prediction model is shown for if this point scoring system is applied on the study group.

In *figure 1* precipitating factors for the 52 hyperkalaemic episodes are depicted; 38% of the episodes of hyperkalaemia were precipitated by periods of dehydration (diarrhoea, fever), change in medication or others. In 62% no precipitating factor for the episode of hyperkalaemia could be found.

DISCUSSION

The favourable outcome of the RALES study has led to the widespread use of aldosterone antagonists in heart failure on top of high doses of ACE inhibitors or ARBs. This has resulted in an increase in the incidence of hyperkalaemia leading to hospitalisation and even death, although the combined therapy has proven to be safe if serum potassium is controlled frequently. In our study we found episodes of hyperkalaemia in 24% of patients. It is important to note that in this retrospective study only living subjects were included. It is possible that we missed patients who had died because of hyperkalaemia. A second shortcoming of this study is that potassium was routinely analysed

Table 1. *Data on study population and cases and controls*

Study population	Total (n=125)	Control (n=95)	Case (n=30)	P value
Binominal variables				
Gender (male)	76 (61%)	55 (58%)	21 (70%)	0.29
HF class (severe)	62 (50%)	40 (42%)	22 (73%)	<0.01
DM	26 (21%)	15 (16%)	11 (37%)	0.02
Creatinine jump	52 (42%)	24 (25%)	28 (93%)	<0.01
Medication use				
Aldosterone antagonist	81 (65%)	60 (63%)	21 (70%)	0.66
ACEi	103 (83%)	77 (81%)	26 (87%)	0.59
ARB	23 (19%)	18 (19%)	5 (17%)	1.00
Diuretics	102 (82%)	75 (79%)	27 (90%)	0.28
β-inhibitor	120 (96%)	91 (96%)	29 (97%)	1.00
Two RAAS-i	82 (66%)	60 (63%)	22 (73%)	0.31
Continues variables mean (±sd)				
Age (years)	65 (±14)	64(±14)	68 (±13)	0.17
GFR (ml/min)	69 (±38)	76 (±40)	45 (±17)	<0.01
LVEDD (cm)	5.70 (±0.9)	5.69 (±1.0)	5.72 (±0.8)	0.87
LVEF (%)	39.2 (±13)	39.4 (±13)	38.4 (±11)	0.74
Potassium (mmol/l)	4.5 (±0.9)	4.1 (±0.4)	5.9 (±0.4)	<0.01
Medication dose (mean pdd/ddd)				
Aldosterone antagonist	0.46 (±0.25)	0.46 (±0.26)	0.45 (±0.22)	0.91
ACE inhibitor	2.26 (±1.38)	2.26 (±1.37)	2.26 (±1.44)	0.98
ARB	1.3 (±0.56)	1.25 (±0.52)	1.5 (±0.71)	0.39

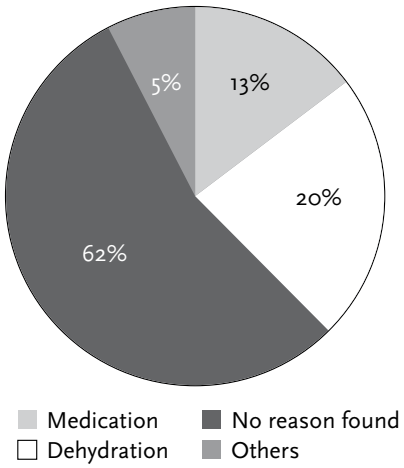
HF class (severe) = New York Health Association (NYHA) class III-IV; DM = diabetes mellitus type 2; creatinine jump = 25% rise and 25% fall of creatinine above 110 µmol/l within three months time; pdd/ddd = prescribed/defined daily dose; ACEi = angiotensin converting enzyme inhibitor; ARB = angio-tensin receptor antagonist; two RAAS-i = use of two potassium-increasing drugs (ACEi, ARB or aldo-a); GFR = glomerular filtration rate; LVEDD = left ventricular end-diastolic dimension; LVEF = left ventricular ejection fraction. p<0.05 is considered statistically significant.

Table 2. *Prediction model hyperkalaemia*

Total points	Control	Case	Total
0	45	3	48
1	20	6	26
2	16	8	24
3	13	9	22
4	1	4	5
Total	*95*	*30*	*125*

Based on the multiple regression a prediction model was developed. A patient with 0 points has NYHA class I or II, GFR >45 ml/min and no diabetes mellitus. Three of these patients developed hyperkalaemia. A patient with 4 points has all risk factors for hyperkalaemia. In one of them no hyperkalaemia was found.

Figure 1. *Precipitating factors for hyperkalaemia*



Precipitating factors for the 52 episodes of hyperkalaemia. In 62% no precipitating factor for the episodes of hyperkalaemia is found. In 38% the possible reason for hyperkalaemia is dehydration, change of medication or others.

every three months, but if a patient had problems, more outpatient clinic visits were scheduled, which led to more frequent blood sampling and hence to bias. This bias is inevitable in a retrospective study. In fact it will even be difficult to circumvent it in a prospective set-up.

In most episodes of hyperkalaemia hospitalisation was not necessary and medication was adjusted to reduce the potassium. In these cases firstly the dose of the aldosterone antagonist was reduced or it was discontinued. Secondly the dose of the ACE inhibitor (or ARB) was reduced. The major risk factor for development of hyperkalaemia was renal failure, which was already recognised in the RALES study, where patients with severe renal failure were excluded (creatinine >221 µmol/l). It is thought that the increase in incidence of hyperkalaemia is partly due to the fact that in daily practice aldosterone antagonists are

also applied in renal failure. In our patients 25 out of 125 patients had a GFR ≤40 ml/min and 14 of these patients received spironolactone. Second, we found that diabetes mellitus is an independent risk factor for hyperkalaemia. This might be due to the fact that insulin is needed for postprandial intracellular disposition of potassium or to the fact that in diabetic patients hyporeninism-hypoaldosteronism is often observed, which leads to poor renal excretion of potassium. As third factor (although not statistically significant) we identified the severity of heart failure as defined by NYHA class III or IV. This can be explained by the fact that kidney perfusion is impaired in severe heart failure, which makes these patients at risk for a transient deterioration in kidney function in periods of (subtle) dehydration. This is confirmed by the fact that in all but one patient there was a transient deterioration in kidney function during the episode of hyperkalaemia, which is probably due to renal hypoperfusion during periods of (subtle) dehydration. Interestingly, a cause for dehydration was only identified in a minority of the patients. Unexpectedly, we did not find that treatment with aldosterone antagonists or renin-angiotensin-aldostonone system (RAAS) inhibitors, or the dose of these drugs, was related to the risk of hyperkalaemia, and change in medication only provoked the hyperkalaemia in 13% of the episodes of hyperkalaemia. In an earlier study on multivariate analysis, these drugs were identified as risk factors (use of spironolactone (OR = 4.18), and use of ACE inhibitors (OR = 2.55)).[8] This may be explained by the fact that in our heart failure outpatient clinic these patients are very intensively monitored, which may lead to withdrawal or lower dosage of these drugs in high-risk patients, because of imminent hyperkalaemia. In our opinion there are two effects. On the one hand aldosterone antagonists and RAAS inhibitors lead to hyperkalaemia. This happens in a vulnerable group of patients with kidney failure, severe heart failure and diabetes. In these patients it is impossible to give the full dose of ACE inhibitors and aldosterone antagonists. This will lead to a lower dosage in this vulnerable group and paradoxically, lower dosage of these drugs in patients at risk for hyperkalaemia and thus lower dosage in patients who have episodes of hyperkalaemia. We feel that this latter effect will be most prominent in a high-risk group that is intensely monitored, i.e. in a tertiary heart failure outpatient clinic. So our results may not be applicable in situations where monitoring is less strict. As in our study the other two independent risk factors identified by these authors are diabetes mellitus (OR = 2.42), and creatinine clearance <40 ml/min (OR = 8.36).[8] We did not find a relationship between the risk of hyperkalaemia and ejection fraction or LVEDD either. In fact, there was no relationship between echocardiographic parameters and NYHA class, which reflects that in the majority of our patients diastolic heart failure is present. It is important to note that despite the identification of the risk factors, the model we have developed based on these risk factors was only a weak predictor of hyperkalaemic episodes. For instance, one out of the five patients who had all risk factors did not develop hyperkalaemia and three out of the 48 patients who had no risk factors still developed hyperkalaemia. Importantly, a clear provoker of the hyperkalaemic episode could only be identified in a minority of patients, and in these cases this was most often due to dehydration. Alteration of drugs or drug dose only caused hyperkalaemia in a very few patients, which is probably due to our policy to check potassium three days after introduction of a drug or dose increment. In conclusion, we identified kidney function, diabetes mellitus and severity of heart failure (as defined by the NYHA class) as independent risk factors for hyperkalaemia. Nevertheless, the model based on these risk factors only gives a weak prediction of hyperkalaemia. In addition, most episodes of hyperkalaemia develop without a clear precipitating factor. These findings imply that heart failure patients in a tertiary reference hospital should be very closely monitored to minimise the risk for hyperkalaemia. This study confirms the notion that these patients should be treated in a specialised heart failure outpatient clinic.[9,10]

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Netherlands
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CASE REPORT

Extremely high serum ferritin levels as diagnostic tool in adult-onset Still's disease

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ABSTRACT

The diagnosis of adult-onset Still's disease (ASD) is difficult to establish due to the nonspecific clinical and laboratory findings. A markedly raised serum ferritin level is a typical finding, although it is not well understood why ferritin levels are extremely high in ASD. We discuss several possible explanations leading to the extremely high levels of ferritin.

KEYWORDS

Adult-onset Still's disease, ferritin

INTRODUCTION

Still's disease is a rare clinical syndrome characterised by the classical triad of high-spiking fever, joint and muscle pain and an evanescent skin rash. The syndrome was first described by George Still in 1897 in children and it was not until 1971 that adult-onset Still's disease (ASD) was recognised as a distinct clinical entity by Bywaters.[1] The present case demonstrates that Still's disease is difficult to diagnose due to nonspecific clinical and laboratory findings except for a markedly increased serum ferritin level.

CASE REPORT

A 52-year old Caucasian man was admitted to the emergency department in March 2006. For four days he had suffered from fever of up to 40°C, generalised myalgia, sternal pain with dyspnoea and a sore throat. The patient's previous history revealed a period with fever, arthralgia, pericarditis and pleuritis in 1986. At that time adult-onset Still's disease was considered and patient recovered spontaneously.

Physical examination revealed a twice-daily spiking fever of up to 40.8°C and bilateral inspiratory crackles on auscultation of the bases of the lung. Further clinical examination was normal, including the absence of lymphadenopathy, skin rash or signs of arthritis.

Laboratory tests at presentation showed a C-reactive protein (CRP) of 175 mg/l, erythrocyte sedimentation rate (ESR) 16 mm/h (maximum 59 mm/h), haemoglobin 10.2 mmol/l, leucocytes 17.3 x 10^9 (93% neutrophils), platelets 129 G/l, normal renal function, lactate dehydrogenase (LDH) 1099 U/l, γ-glutamyltransferase (γGT) 67 U/l, serum aspartate transaminase (ASAT) 77 U/l, serum alanine transaminase (ALAT) 34 U/l, total bilirubin 30 μmol/l, ferritin 75,500 μg/l, iron (Fe) 6.5 μmol/l, iron saturation 26% and creatinine phosphokinase (CK) 33 U/l. Several serological results were negative (Rf, anti-CCP, ANA, ANCA). On the day of admission chest X-ray and electrocardiography were normal. Because an infectious disease was suspected, the patient received broad-spectrum antibiotics (cefuroxim and gentamicin) after blood, urine and sputum cultures had been taken. During his stay in hospital, the spiking fever persisted, while the patient developed signs of bilateral pleural effusion on chest X-ray. Bronchoscopy showed no signs of inflammation. Echography of the abdomen revealed only a slightly enlarged spleen. Electrocardiography showed atrial fibrillation with a normal ventricular response. Echocardiogram showed no abnormalities, especially no pericardial fluid. Blood, urine and sputum cultures were repeatedly negative.

A flare-up of adult-onset Still's disease was established, especially based on the clinical symptoms and the markedly increased levels of serum ferritin (75,500 μg/l). Antibiotic therapy was replaced by nonsteroidal anti-inflammatory agents. Because this provided little clinical effect, corticosteroids were added (prednisone 40 mg/

day). The patient's condition rapidly improved and he was discharged. Despite the clinical improvement and decrease in CRP and ferritin levels, the liver function tests rose to following levels: bilirubin 32 μmol/l, γGT 541 U/l, AF 329 U/l, ASAT 116 U/l and ALAT 526 U/l (*figure 1*). Corticosteroids were continued and after two weeks the liver tests has largely returned to normal.

Figure 1. *Laboratory findings*

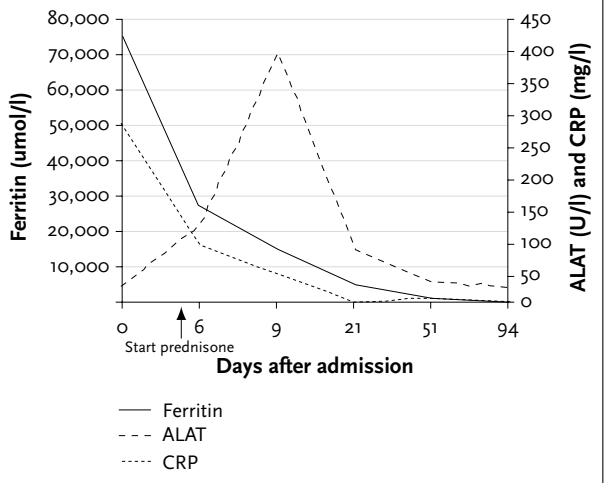

Table 1. *Diagnostic criteria for adult-onset Still's disease*[2]

Five or more of the diagnostic criteria listed below, of which two must be major criteria	
Major criteria	Fever ≥39°C (one week or longer)[+]
	Arthralgia and/or arthritis (two weeks or longer)[+]
	Nonpruritic, pink, macular or maculopapular rash, usually during febrile episodes
	Leucocytosis (>10,000 μmol/l, >80% neutrophils)[+]
Minor criteria	Pharyngitis[±]
	Lymphadenopathy and/or splenomegaly[±]
	Liver involvement (raised serum transaminases and/or lactate dehydrogenase)[+]
	Negative rheumatoid factors and antinuclear antibodies[+]
Exclusion criteria	Infectious diseases
	Malignant diseases
	Rheumatological conditions

[+]**Present in our patient;** [±]**partly present in our patient.**

DISCUSSION

In our patient ASD was diagnosed. This disease is frequently a diagnosis by exclusion, which may result in delayed intervention and unnecessary diagnostic procedures in many cases.

Several sets of classification criteria have been developed. The most frequently used criteria are those of Yamaguchi *et al.* (*table 1*).[2] Besides these criteria other manifestations may be present, including hepatomegaly and/or splenomegaly and/or cardiac and pulmonary features. Cardiac involvement includes pericarditis and less frequently myocarditis and pulmonary involvement is characterised by pleuritis and pulmonary infiltrates. Our patient in this case satisfied the criteria for ASD according to Yamaguchi (*table 1*) and suffered from pleuritis as well.

The aetiology of ASD is still unclear, although some studies have suggested a role for viral or bacterial triggers such as rubella, Epstein-Barr virus, cytomegalovirus and *Mycoplasma pneumoniae* in the pathogenesis of ASD.[3,4] The peak age of onset is usually between 15-25 and 36-46 years, and both sexes are equally affected.[5]

A raised ferritin level is often found during episodes of adult-onset Still's disease.[6] Serum ferritin levels are markedly increased during active disease and return to normal values during remission. Nevertheless, a serum ferritin level as found in this patient is exceptional and has been described in few other cases.[7,8] Other conditions in which ferritin levels may be elevated are infections, malignancies (leukaemia, lymphomas), liver diseases and haemochromatosis. However, in these conditions serum ferritin concentrations rarely exceed values of >3000 μg/l. Besides in adult Still's disease, serum ferritin levels of >10,000 μg/l have only been described in severe liver damage, after multiple blood transfusions or in the haemophagocytic syndrome. So the ferritin level may be an important diagnostic tool in the diagnosis of Still's disease and should be therefore included in the classification criteria.

The cause of the extremely high ferritin concentrations in adult-onset Still's disease remains unclear. Some studies suggest that ferritin is released into the plasma due to liver cell necrosis.[9] In this case, ferritin levels were already markedly increased before liver function tests started to rise, making this explanation not probable. Others have found increased production of ferritin mediated by several cytokines, mainly interleukin IL-1α, IL-1β, IL-6 and TNFα.[10] This so-called acute phase response seems to stimulate the synthesis of ferritin, although it does not explain why the ferritin levels in ASD are usually much higher than those found in patients with other inflammatory diseases and comparable levels of CRP. Fautrel *et al.* reported on a decrease in glycosylated ferritin, an isoform of ferritin, in ASD compared with other inflammatory conditions.[11] In healthy subjects, 50 to 80% of ferritin is glycosylated, in inflammatory diseases the glycosylated part drops to 20 to 50%, while in ASD the glycosylated part of ferritin is often <20%. Decreased clearance from the plasma of nonglycosylated ferritin by the histiocyt-macrophage system may partly explain the increased levels of ferritin.[12]

When combined with a fivefold serum rise in ferritin, glycosylated ferritin <20% reaches a sensitivity of 43% and a specificity of 93% to diagnose ASD.[13] Kirino *et al.* suggested ferritin synthesis to be stimulated by haeme-oxygenase-1, an inducible haeme-degrading enzyme.[14] Ferritin synthesis is stimulated by Fe^{2+}, which is a product of haeme degradation. Haeme oxygenase-1 is expressed on macrophages and endothelial cells in response to various forms of stress.

In conclusion, although the origin of the high ferritin concentration remains unclear, an extremely high serum ferritin level in addition to classification criteria makes it much easier to diagnose ASD.

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CASE REPORT

Life-threatening *Pneumocystis jiroveci* pneumonia following treatment of severe Cushing's syndrome

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ABSTRACT

We describe two patients with a severe Cushing's syndrome due to ectopic production of ACTH. Both patients developed a life-threatening *Pneumocystis jiroveci* pneumonia (PCP) shortly after treatment of the hypercortisolism was started by means of inhibition of production of glucocorticoids and glucococorticoid receptor blockade. We presume that the restored immune response elicited the clinical symptoms of the opportunistic, previously subclinical *Pneumocystis jiroveci* infection. The immunocompromised state and the delicate glucocorticoid balance in patients with a severe Cushing's syndrome necessitate a specific diagnostic and therapeutic approach.

KEYWORDS

Cushing's syndrome, opportunistic infections, treatment

INTRODUCTION

Cushing's syndrome is a well-known but nevertheless rare syndrome with an incidence of 0.7 to 2.4 per million population per year and a prevalence of 39.1 cases per million, iatrogenic cases not included.[1,2] Cushing's syndrome results from lengthy and inappropriate exposure to excessive concentrations of circulating free glucocorticoid. In most cases Cushing's syndrome is adrenocorticotropin hormone (ACTH) dependent, originating either from a pituitary ACTH-secreting tumour (Cushing's disease) or, less frequently, from nonpituitary tumours secreting ectopic ACTH. Of all patients with endogenous Cushing's syndrome 9 to 18% have ectopic ACTH production.[3,4] We describe two patients with opportunistic respiratory infections due to extremely high cortisol levels because of ectopic ACTH production. In both patients the symptoms developed shortly after the treatment of the hypercortisolism was started.

CASE REPORT 1

A 62-year-old woman presented with complaints of hypertension, hirsutism, oedema of the tongue, a moon face and visual disturbances. These complaints had already been present for three years but varied in intensity. She had lost 10 kg in weight over the last two months. Moreover, during the last weeks she also developed muscle weakness in the lower extremities. She was found to have hypokalaemia. She also had a new-onset diabetes mellitus. ACTH (296 ng/l, normal <46 ng/l), cortisol (1945 nmol/l, normal <800) and urine cortisol (51,414 nmol/24 h, normal <270 nmol/24 h) were markedly elevated. A pituitary adenoma was not seen with gadolinium-enhanced magnetic resonance imaging (MRI). She was referred to the Department of Endocrinology in our hospital under the suspicion of an ectopic ACTH-producing tumour. Inferior sinus petrosus sampling confirmed the already supposed absence of pituitary ACTH production. A computerised tomography (CT) scan of the abdomen and thorax revealed a solid mass of 1.5 cm in the left upper lobe of the lung. The pulmonary lesion showed an increased uptake on (18)F-DOPA-PET. This

made a pulmonary carcinoid very likely. Spironolactone and mifepristone (400 mg) were started as dual receptor blockade, in anticipation of pulmonary surgery.
A few days later she complained of dyspnoea. Bilateral infiltrates were seen on the chest X-ray. She was admitted to the intensive care unit because of severe hypoxia. A bronchial lavage was carried out but was negative for *Pneumocystis jiroveci* or other pathogens. Nevertheless, high-dose trimethoprim-sulphamethoxazole (3 x 1920 mg) was started because of the strong clinical suspicion of *Pneumocystis jiroveci* pneumonia (PCP). Dyspnoea, hypoxia and chest X-ray improved. She did not need ventilatory support and was transferred back to the ward. Few weeks later the patient underwent a left upper lobectomy. Histology indeed showed a carcinoid of the lung with evidence of ACTH production. Cortisone acetate substitution was necessary for six months following surgery. Afterwards endogenous cortisol production was sufficient, with normal suppression after repeated dexamethasone inhibition. She has been free of symptoms for one year now.

CASE REPORT 2

A 57-year-old woman was well until January 2006, when she noticed malaise. Hypertension was found in May 2006 for which she was referred to hospital. Cushing's syndrome was diagnosed. She was found to have multiple masses in the liver and a solid mass in the tail of the pancreas by CT scan. Percutaneous liver biopsy revealed an undifferentiated non-small-cell carcinoma with some neuroendocrine characteristics. Laboratory examinations revealed marked elevations of plasma ACTH (318 ng/l, normal <46 ng/l), cortisol (2371 nmol/l, normal <800) and urine cortisol (294,306 nmol/24 h, normal <270 nmol/24 h) and hypokalaemia. Her hypertension required medication. Insulin was started because of a diabetes mellitus de novo. She was referred to the Department of Endocrinology in our hospital in June 2006. A diagnosis of severe Cushing's syndrome was made, due to ectopic ACTH production, probably from a primary endocrine tumour of the pancreas with liver metastases. Palliative, but directly life-saving bilateral adrenalectomy was contemplated because of the extremely high cortisol level. Curative surgery was impossible but the usual slow growth of neuroendocrine tumours made this attempt worthwhile.
Symptomatic treatment was started with ketoconazole, shortly afterwards followed by additional dual receptor blockade with 400 mg mifepristone and spironolactone. Also trimethoprim-sulphamethoxazole prophylaxis (960 mg on alternate days) was started as *Pneumocystis jiroveci* pneumonia prophylaxis. Nevertheless, she became dyspnoeic two days later and was transferred to the ICU. Physical examination on admission showed a typical Cushingoid appearance with moon face, alopecia, muscle weakness, striae and central obesity. She was tachypnoic (30 breaths/min) and had a peripheral oxygen saturation of 80% with a 100% O_2 non-rebreathing mask. She was haemodynamically stable. Chest X-ray revealed bilateral alveolo-interstitial opacities. Before further diagnostic procedures could be performed she had to be intubated and mechanically ventilated. A bronchial lavage was carried out, which revealed *Pneumocystis jiroveci*. Afterwards trimethoprim-sulphamethoxazole was given in a therapeutic dose (3 dd 1920 mg iv). Mifepristone was stopped and glucocorticosteroids (hydrocortisone 400 mg/24 h) were started because of hypotension. The pulmonary symptoms improved.
For better control of the cortisol levels she underwent the planned bilateral adrenalectomy five days later. Large tumours in the liver and pancreas were found during the laparotomy, as well as a peritonitis carcinomatosa. A biopsy from the peritoneum showed a neuroendocrine tumour. Adipositas and muscle weakness made weaning difficult but ultimately she could be extubated. She had a short but much valued time with her family before she died, probably due to tumour progression.

DISCUSSION

We describe two patients with Cushing's syndrome and very high cortisol levels due to an ectopic ACTH production. One patient had a proven PCP; the other patient was clinically very suspect for a PCP. Both patients responded well to specific PCP therapy. Opportunistic infections after external glucocorticoids are well known, but opportunistic infections in patients with endogenous cortisol overproduction are less common. However, as early as in 1952, infections and wound healing problems in 17 of 33 patients with Cushing's disease, all untreated for their Cushing's syndrome, were described.[5] Graham described six patients with severe endogenous Cushing's syndrome and opportunistic infections.[6] He showed that patients with Cushing's syndrome have the same spectrum of infections as patients treated with pharmacological doses of corticosteroids. The risk of an opportunistic infection in Cushing's syndrome is related to the cortisol level.[6,7] An opportunistic infection is therefore less likely to occur in patients with pituitary Cushing's disease than it is in patients with higher levels of cortisol overproduction from adrenal tumours or due to ectopic ACTH secretion. But still there is an increased risk, also for pituitary gland related Cushing's disease.[8,9] *Cryptococcus neoformans, Aspergillus fumigatus, Nocardia* spp and *Pneumocystis jiroveci* are the most frequently found pathogens.

Immunocompromised hosts other than HIV patients can have a low *Pneumocystis* load explaining the negative broncoalveolar lavage in the first patient.[10,11] Interestingly, in both patients the clinical symptoms occurred shortly after almost total blockade of cortisol activity by the use of mifepristone. This has been described previously. The reconstituted immune response might be responsible for this.[8] Apparently there is a delicate balance between the immune system of the patient and the pathogen that can be disturbed by treatment. This is also illustrated by the treatment of severe PCP in HIV patients. In these patients PCP treatment is combined with glucocorticosteroids to avoid a life-threatening immune response.[12]

Mifepristone is used to induce medical abortion. It has also been suggested to potentiate infections in this setting. The mechanism behind this might be the innate immunity.[13] This might be a complementary or alternative explanation for the sequence of events seen in our patients. The incidence of infection after mifepristone is, however, very low and it is a completely different population, so this is not very likely. Enzyme inhibitors as ketoconazole have a rapid onset of action but these drugs are not effective enough in severe Cushing's syndrome. Mifepristone is a highly potent antagonist of glucocorticoid and progesterone receptors, especially suited for use in severe Cushing's syndrome as temporary medical therapy. Indeed both patients became insulin independent after mifepristone therapy started. Titration of the mifepristone dose is difficult because the effect of mifepristone, as a receptor blocker, cannot be quantified by the cortisol level itself. Insulin dependence and blood pressure can both be measured for glucocorticoid activity. However, excess inhibition is less easily measurable and in case of doubt mifepristone should be stopped and cortisol be substituted. In addition, a mineralocorticoid receptor antagonist such as spironolactone is usually necessary to control hypokalaemia.[14]

Definitive therapy is surgical extirpation of the ACTH-producing tumour, if feasible. Bilateral adrenalectomy may be a useful palliative therapy in case of metastasised disease.[15,16] In conclusion, high endogenous glucocorticoid levels are immunosuppressive. Glucocorticoid receptor blockade by mifepristone is a powerful temporary medical treatment, awaiting definitive surgical therapy. However, this might elicit clinical symptoms of a previous subclinical *Pneumocystis jiroveci* infection justifying at least prophylactic and maybe even therapeutic doses of trimethoprim-sulphamethoxazole. Titration of the optimal level of corticoid activity is a clinical challenge in these critically ill patients.

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Netherlands
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PHOTO QUIZ

Abdominal pain with unexpected pulmonary consequences

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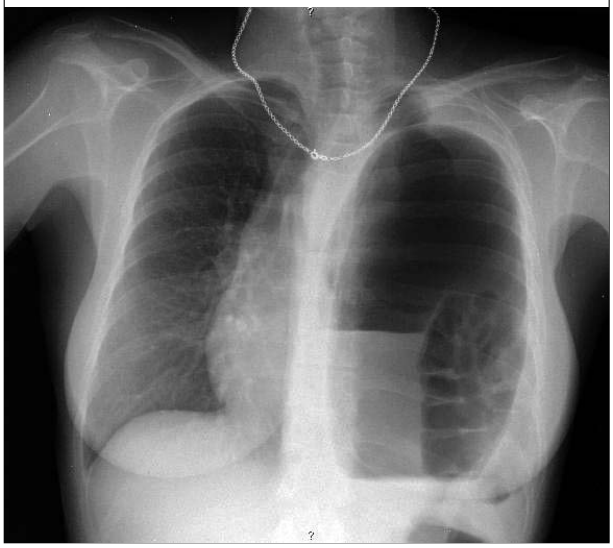
CASE REPORT

A 42-year-old woman presented to the emergency room with acute, colicky epigastric pain causing difficulty in breathing. She was nauseous but did not vomit. For one month she had suffered several similar attacks, generally after meals. Her medical history was unremarkable. There was no previous (abdominal) trauma.

Physical examination revealed no abnormalities besides mild discomfort on palpation of the epigastric region. A routine blood examination and abdominal ultrasound were normal. Gastroscopy revealed no mucosal abnormalities but passing the pylorus proved abnormally difficult. Immediately after the endoscopy the patient experienced severe chest pain and dyspnoea. Breath sounds over the left lung were absent. A chest X-ray was performed (*figure 1*).

Figure 1. *Chest X-ray*



WHAT IS YOUR DIAGNOSIS?

See page 220 for the answer to this photo quiz.

Netherlands
The Journal of Medicine

PHOTO QUIZ

Neck swelling following a vigorous neck massage

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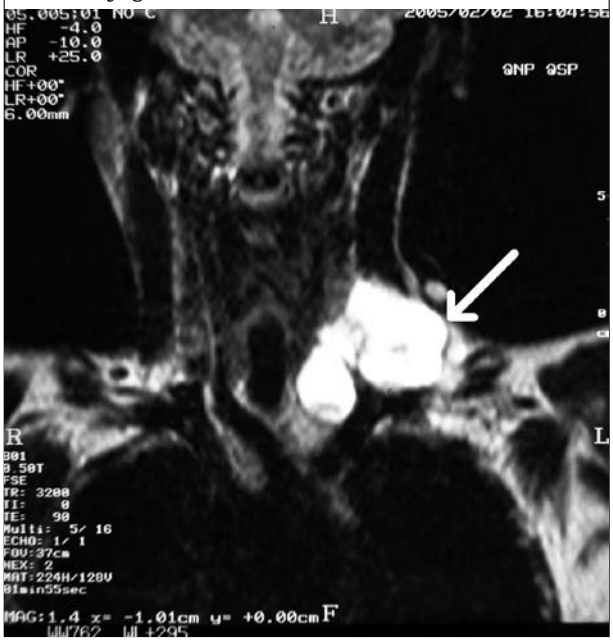
CASE REPORT

A 19-year-old man was admitted with a left supraclavicular mass that had been present for three days, and had started two days after undergoing vigorous massage of the neck by a professional masseur. The mass had increased progressively in size over the ensuing three days prior to attendance at the hospital, but there were no other relevant symptoms, and specifically no dyspnoea or dysphagia. On physical examination, there was a 6 x 5 x 6 cm painless and fluctuant mass in the left supraclavicular fossa, overlapping the sternocleidomastoid muscle anteriorly, and no coexisting adenopathy or additional neck mass. Ultrasonography of the neck revealed a fluid mass suggesting a venous haemorrhage within the neck musculature, whereas magnetic resonance imaging (MRI) demonstrated a 6 x 5.5 x 6 cm cystic mass located in the supraclavicular region between the carotid artery and internal jugular vein (*figure 1*). Percutaneous needle aspiration was performed, and the aspirate found to be milky macroscopically. Biochemical analysis of the fluid revealed the following: cholesterol 90 mg/dl, triglycerides 2400 mg/dl, and white blood cell count 3200 cells/mm^3 (80% lymphocytes).

Figure 1. *MRI: A cystic mass located in the supraclavicular region between the carotid artery and internal jugular vein*



WHAT IS YOUR DIAGNOSIS?

See page 221 fot the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (ON PAGE 218)

ABDOMINAL PAIN WITH UNEXPECTED PULMONARY CONSEQUENCES

DIAGNOSIS

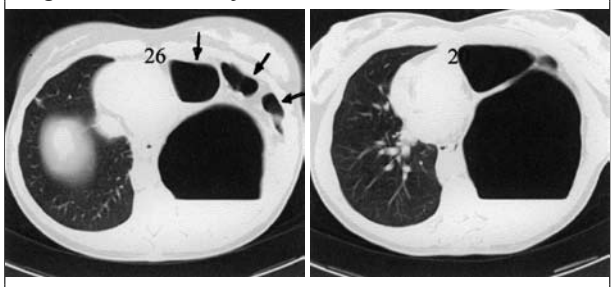
Chest X-ray showed a tension gastrothorax. There is deviation of the heart to the right. The air-fluid level and visable haustration represent stomach and colon in the left hemithorax. Computed tomography (CT) scan (*figure 2*) showed a diaphragmatic hernia through which the stomach and part (splenic flexure) of the colon herniated into the chest cavity causing the colic. The air insufflated during the gastroscopy caused the tension gastrothorax. Treatment consisted of desufflation of the stomach through a nasogastric tube performed slowly to prevent pulmonary oedema. Several days later she underwent surgery: the spleen, stomach and part of the left colon were found to be intrathoracic. Splenectomy was performed, the stomach and colon repositioned intra-abdominally after which the diaphragm could be repaired. Recovery was uneventful.

Tension gastrothorax has been described in children due to a congenital diaphragmatic defect or Bochdalek hernia.[1-4] Tension gastrothorax develops when the stomach herniated through a Bochdalek hernia is distended by trapped air.[1] In adults a nontraumatic gastrothorax through a Bochdalek hernia is very rare and to our knowledge only a few cases have been described.[5,6] Tension gastrothorax in adults usually develops after traumatic rupture of the diaphragm and can be misdiagnosed as tension pneumothorax.[7-9] Only one case of tension gastrothorax as a result of traumatic diaphragmatic hernia in children has been reported.[10]

The diagnosis is made by a high level of clinical suspicion, chest X-ray reveals an air-fluid level and an elevation of the diaphragm, and a computerised tomography is useful in assessing the diaphragm and establishing the positions of the various intra-abdominal organs.[10]

Decompression of the distended stomach should first be attempted via a nasogastric tube.[10] This should be performed slowly to prevent pulmonary oedema.[11] If this fails, decompression must be achieved either by needle puncture or by chest tube insertion into the stomach.[1] Definitive management is surgery when the patient has been stabilised.[10]

Figure 2. *CT scan of the thorax*



Normal lung tissue on the right. Deviation of the heart to the right. The left hemithorax shows several cavities filled with air, the largest with an air-fluid level is the stomach, the others are part of the left hemicolon (arrows). Normal lung tissue is absent, only compressed lung tissue dorsomedial of the stomach (arrowhead).

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ANSWER TO PHOTO QUIZ (ON PAGE 219)

NECK SWELLING FOLLOWING A VIGOROUS NECK MASSAGE

DIAGNOSIS

The thoracic duct is the main collecting vessel of the lymphatic system. It is the common trunk of almost all the lymphatic vessels of the body (*figure 2*). Injuries of the thoracic duct at neck dissection occur in 1 to 6% of cases, with the majority of the cases being on the left side, but they may also result from penetrating and blunt trauma.[1] Injury to the thoracic duct resulting in leakage of lymph presents in the form of a chylous fistula, chylothorax, or lymphocele. The diagnosis of this case was considered to be a cervical lymphocele. The lymphocele is defined as a circumscribed fluid collection without an epithelial lining and is attributable to persistent leakage into a confined space.[2] Ultrasound, computed tomography scan and MRI are useful in delineating the precise extent of the lesion. Aspiration of the cyst is important for the diagnosis in that it should reveal a milky fluid owing to its fat content, the concentration of which should range between 0.4 and 4% depending upon the diet.

In most cases treatment is conservative and includes bed rest, head elevation, continuation of closed drainage, dietary restriction and external pressure.[3] Although conservative treatment is successful in controlling the majority of lymph leaks, surgery is found to be necessary in persistent chylous fistula or when fistula drainage exceeds 500 to 600 ml per day.[4] The size of the lesion, failure of conservative management, and the presence of compression symptoms are the other indications for surgical treatment. In this case the treatment, which was conservative, included serial percutaneous aspiration (a total 70 ml over seven days), a low fat diet and repeated pressure dressings. Examination one month later showed no evidence of a recurrence of the mass, and an MRI performed concurrently also showed complete regression of the pathology (*figure 3*). One year after treatment, the patient was asymptomatic and showed no evidence of disease or recurrence of the lymphocele.

Figure 2. *The thoracic duct*

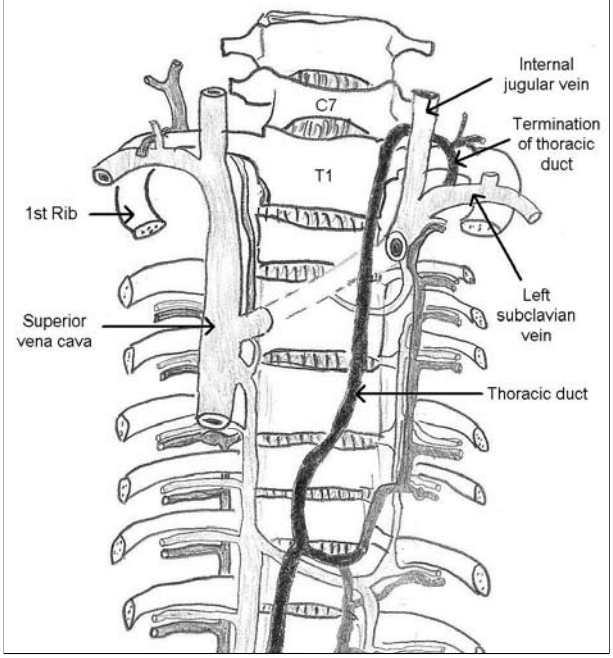
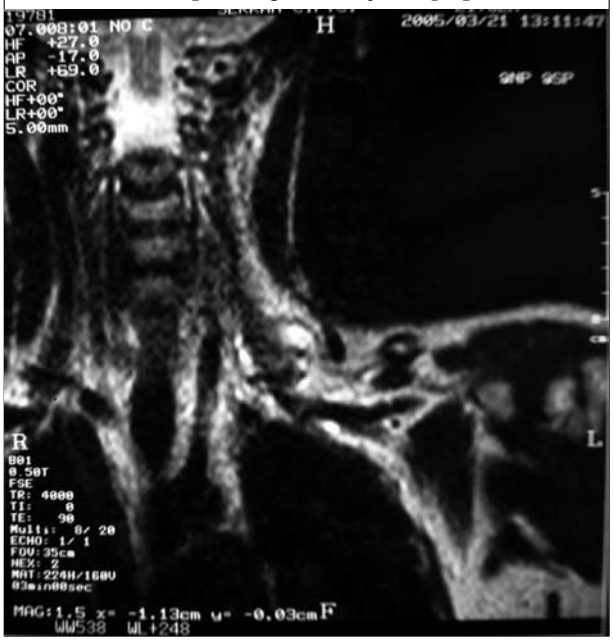


Figure 3. *An MRI was performed after one month and showed complete regression of the lymphocele*



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Fatal disseminated toxoplasmosis after liver transplantation: improved and early diagnosis by PCR

KEYWORDS

Liver transplantation, real-time PCR, toxoplasmosis

Disseminated toxoplasmosis after liver transplantation is a rare, but often fatal, event.[1] The high mortality rate is generally due to a delay in diagnosis and initiation of therapy.[2] The classical diagnosis of toxoplasmosis based on serological tests can be unreliable in transplant patients. Therefore, the diagnosis is usually based on the direct demonstration of the parasite in tissues or biological fluids.[2,3] However, these techniques are time-consuming and lack sensitivity. We report a case of disseminated toxoplasmosis after liver transplantation, evaluating the use of quantitative polymerase chain reaction (PCR) as an early diagnostic tool.

A 36-year-old Dutch male underwent an orthotopic liver transplantation on 29 October 2002. The immunosuppressive regimen included mycophenolate mofetil, prednisone and cyclosporine. At 21 days post-transplantation the patient presented with fever. Cytomegalovirus infection was diagnosed and treated with ganciclovir. Despite this therapy, the fever persisted, with ascitis and splenomegaly. Antibiotics were started because of suspicion of peritonitis, although cultures remained negative. In the following days pancytopenia and renal insufficiency developed. At day 44, the patient developed acute respiratory failure. A bronchoalveolar lavage (BAL) was performed and a few hours later the patient died due to cardiac arrest. Microscopic examination of the BAL fluid revealed several *Toxoplasma* tachyzoites. *Toxoplasma* serology remained negative. A real-time PCR with *T. gondii* specific primers and detection probe which amplify and detect a 100-bp fragment within the *T. gondii* B1 gene[4] was performed on DNA isolated from plasma and other clinical samples. For quantification, a tenfold dilution series of *T. gondii* DNA from a known number of parasites was included in the amplification run. Simultaneous isolation, amplification and detection of a standard amount of phocid herpes virus was used as an internal control of inhibition.[5] *Toxoplasma* DNA was detected in the BAL fluid and in lung, pleura, liver and spleen samples obtained after obduction. Plasma samples showed increasing levels of *Toxoplasma* DNA, first detectable on day 37 after transplantation (*figure 1*). In retrospect, the donor had serological evidence of a prior *Toxoplasma* infection, suggesting *Toxoplasma* transmission via an infected allograft. In conclusion, real-time PCR on plasma proved to be a simple, rapid, and highly sensitive method to diagnose disseminated toxoplasmosis. Since early initiation of specific anti-*Toxoplasma* therapy is a critical prognosis factor, highly sensitive PCR methods that can be applied directly to plasma samples could be of great help in cases of unexplained fever in immunocompromised recipients.

ACKNOWLEDGEMENTS

Dr J. Schinkel, Department of Microbiology, Academic Medical Centre Amsterdam, the Netherlands is acknowledged for retrospectively determining the *Toxoplasma* serology status of the donor.

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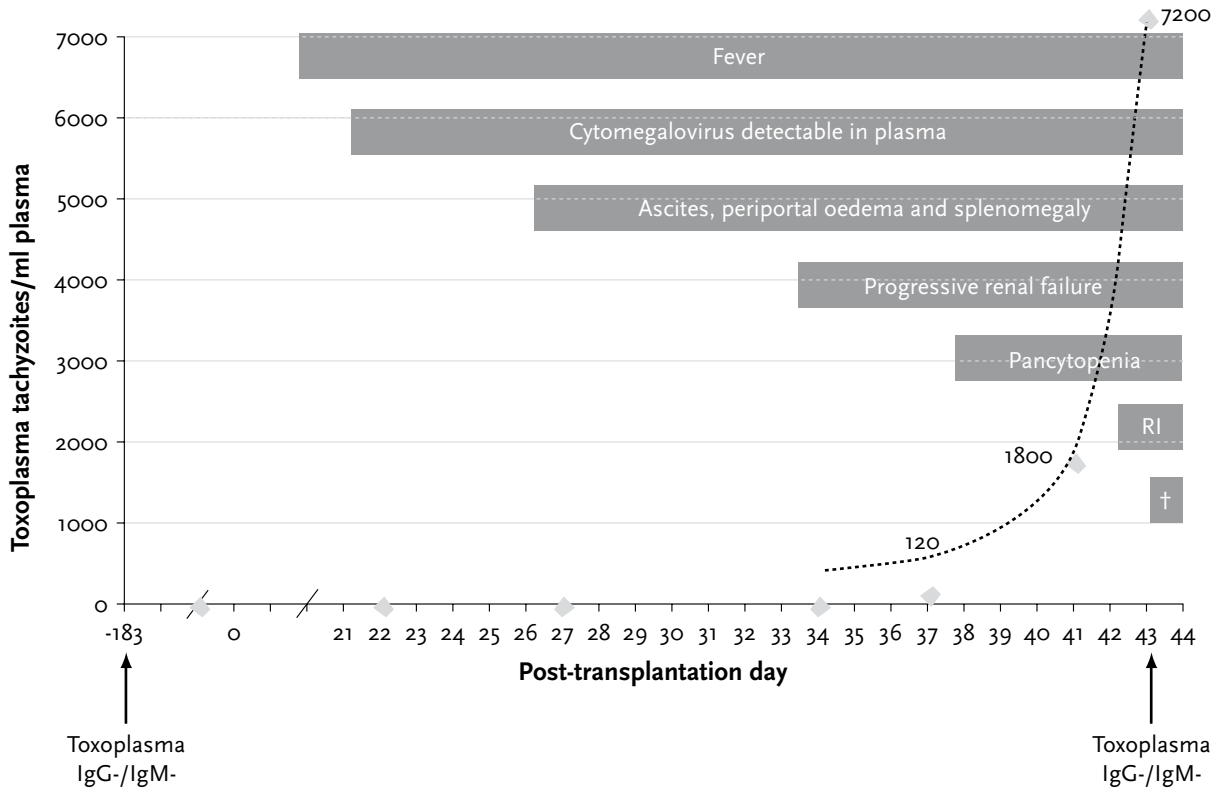
Figure 1. *Serology and quantitative Toxoplasma gondii real-time PCR test analysis of sequential plasma samples from a patient with disseminated toxoplasmosis after orthotopic liver transplantation*



RI = respiratory insufficiency. †Death.

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EDITORIAL	
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REVIEW	
Recurrent erysipelas despite antibiotic prophylaxis: an analysis from case studies	183
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Total	**1616**

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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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