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The largest Q fever outbreak ever reported

P. Speelman

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Since 2007, the Netherlands has experienced the largest Q fever outbreak ever reported in the literature. From 2007 till today approximately 4000 people have been affected and at least 14 of these patients, nearly all of them with severe underlying conditions, have died. The clinical details of the patients who died are described by Kampscheur et al. in this issue of the Journal.¹

It all started in May 2007 when medical specialists in a hospital in the southern part of the Netherlands and a general practitioner in the same region reported a substantial number of patients with pneumonia not responding to amoxicillin, whereas patients did well on moxifloxacin. Soon it became clear that these pneumonias were caused by Coxiella burnetii. During 2007, 168 patients were reported. Although an association with intense goat farming was already suggested in 2007² and experts warned that the outbreak could recur, a limited number of preventive measures were taken. The experience that earlier reported outbreaks were usually limited in time and did not recur in the following years may have played an important role. In 2008, however, the epidemic recurred and 1000 patients were reported. This prompted more preventive regulations. In June 2008, Q fever finally became a notifiable disease for the veterinary sector as well. A ban on the spread of manure from farms with Q fever was issued and (voluntary) vaccination of non-pregnant goats on dairy goat farms in the region was advised. In 2009 compulsory hygiene measures in farms with more than 50 goats or sheep were instituted and compulsory vaccination of non-pregnant goats on dairy goat farms in the region was advised. In 2009 compulsory hygiene measures in farms with more than 50 goats or sheep were instituted and compulsory vaccination of goats and sheep in farms with more than 50 animals was ordered. Later that year testing for Coxiiella burnetii on bulk milk was started and transport of cattle was prohibited. In December 2009 breeding on infected farms was prohibited. After a lot of political pressure and a critical documentary on national television, it was decided to start culling more than 50,000 pregnant goats on infected farms. In January 2010 compulsory vaccination for all goats and sheep were instituted. In 2010 up to 3 November, 492 patients with Q fever (not confirmed yet) have been reported. Since several measures have been instituted simultaneously it is difficult to speculate which measures have been most effective. It is very likely that the culling of all pregnant goats has had an important impact. It is clear, however, that most interventions were issued too late. Retrospectively, we know that Q fever was already a problem in the veterinary sector in 2005 and 2006 but that this knowledge was not communicated to the human health sector.

Approximately 4000 people were affected. Among the admitted patients fever and flu-like symptoms with headache and cough were the most important symptoms. Most inpatients had an infiltrate on the chest X-ray. CURB scores on admission day were 0 +/- 1, indicating mild pneumonia.³ As reported by Delsing et al. in this issue of the Journal³ acute Q fever may be followed by a chronic fatigue syndrome. A recently published case-control study among 54 patients who contracted Q fever in 2007 reported severe fatigue, one year after acute Q fever, in over 50% of the cases compared with 26% of controls.³ Chronic Q fever may develop in 1 to 2% of patients after acute Q fever. Although endocarditis is a feared complication, in the Netherlands infected aneurysms and infected vascular prosthesis were more frequently reported and therefore screening of patients with aortic aneurysms has been advocated.³ Delsing et al. estimate that over the last three years 40 to 50 cases of chronic Q fever have been diagnosed.

A French study in pregnant patients with Q fever reported obstetric complications in 80% of patients, compared with 44% of patients who were treated with co-trimoxazole.³ Other studies, however, did not find an association between seropositivity for Coxiiella burnetii and adverse pregnancy outcome. A retrospective study in the Netherlands did not find a correlation between seropositivity during early pregnancy and adverse pregnancy outcome.³ A randomised controlled trial has started this year aimed at providing conclusive data on the need to screen pregnant women in high incidence areas.

Recently the Health Council of the Netherlands has advised the Minister of Health to provide the only available vaccine (Q-VAX, developed and registered in Australia) for...
certain high risk groups (http://www.gezondheidsraad.nl/nl/adviezen/vaccinatie-van-mensen- tegen-q-koorts-eerste-advies). These people should be tested serologically and with a skin test since previous exposure to *Coxiella burnetii* may lead to serious side effects. Since the data on efficacy and safety are limited the vaccine should not be administered to pregnant women and people below the age of 15 years.

An extremely important question still remains unanswered. How is it possible that so many people became infected and that an epidemic of historic proportions could develop?

Factors that may have played a role include the high number of goats in highly populated areas, environmental factors and the possible introduction of a more virulent strain of *Coxiella burnetii*. The number of dairy goats in the Netherlands has increased from 98,000 in 2000 to 231,000 in 2009. The number of farms with more than 100 goats increased from 33 to 58. In the Netherlands there are hardly any goat farms without many people living in the proximity of the farm. From earlier studies and also from a recent study it is known that the relative risk for people to contract Q fever is directly related to the distance between the infected farm and their house. Environmental factors may also play a role, e.g. weather conditions. In 2007 we had a long dry period without rain. Recently Hunink *et al.* reported a correlation between vegetation density and higher groundwater levels and lower transmission of Q fever from infected farms in various regions of the Netherlands (RIVM report no. 90, 1 January 2010). Studies on isolated strains of *Coxiella burnetii* are ongoing. The Central Veterinary Institute has reported that one MLVA type prevails on many dairy goat farms in the southern part of the Netherlands, possibly indicating clonal spread in this area. In a new project whole genome sequencing will be used to distinguish between *Coxiella* bacteria from different sources.

Infectious diseases remain important. New infectious diseases appear and old infectious diseases re-emerge. Approximately 75% of the re-emerging infectious diseases are zoonoses. In a densely populated country with an extremely high concentration of farm animals suitable systems have to be available for timely recognition of emerging zoonotic diseases in humans and farm animals. The recently published report ‘Emerging zoonoses: early warning and surveillance in the Netherlands’ is an important step on the road to an effective human-veterinary early warning system (EMZOO rapport: Emerging zoonoses: Early warning and surveillance in the Netherlands; http://www.rivm.nl/bibliotheek/rapporten/330214002.pdf)

**References**

Q fever in the Netherlands from 2007 to 2010

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Abstract

Since 2007, the Netherlands is faced with the largest outbreak of Q fever ever reported. In the last four years, over 4000 cases have been reported. The course of the epidemic and possible factors associated with this sudden surge in cases of Q fever is described and the preventive measures in the veterinary sector and the outbreak management of this unique epidemic are summarised. Finally, the latest data on clinical presentation and diagnostic and therapeutic dilemmas of Q fever in the Netherlands are reviewed.

Keywords

Coxiella, Q fever, the Netherlands

Introduction

Since 2007, the Netherlands is faced with the world’s largest outbreak of Q fever with over 4000 notified cases. Q fever is caused by Coxella burnetii, a small, Gram-negative obligate intracellular bacterium, phylogenetically related to Legionellales. Transmission occurs through inhalation of infected aerosols. The reservoir consists mainly of dairy goats and sheep, but excretion of Coxella has also been described in other cattle and rodents. Small outbreaks associated with par-turient pets such as cats and dogs have also been reported. The animals shed the bacterium in urine and faeces, and in very high concentrations in birth by-products. C. burnetii is extremely infectious as was illustrated by an experiment demonstrating that inhalation of a single bacterium could cause seroconversion in humans. It was classified as a category B bioterrorism agent.

Epidemiology and Control of Q fever in the Netherlands

The epidemic in the Netherlands is concentrated in the southern part of the country, in the provinces of Brabant, Gelderland and Limburg, although increasing numbers are being reported from the Northern provinces. A seroprevalence study performed on samples that were collected in a national immunisation survey in 2006 (PIENTER-2) showed a low seropositivity rate for Q fever of 2.4% just before the outbreak, indicating that the epidemic in 2007 was indeed newly emerging and did not merely reflect heightened awareness of physicians for Q fever. Already in 2007, an association with intense goat farming in the region was suggested. In 2008, a large human cluster of Q fever in an urban area was clearly linked to a dairy goat farm with a Q fever related abortion episode a few weeks before the first human cases presented. The high relative risk (31.1 [95% CI 16.4 to 59.1]) to contract Q fever when living within a 2 km radius of a dairy goat farm compared with persons living more than 5 km away supported this hypothesis. Although most cases seem to be related to dairy farming, transmission from non-dairy sheep has also been described in a small epidemic of at least 28 cases among patients and staff of a psychiatric institution. Besides proximity to urbanisation, multiple other environmental factors are involved in the transmission of Q fever from infected farms. Hunink and colleagues showed a correlation between higher vegetation density and higher groundwater levels and lower transmission of Q fever from infected farms in various regions in the Netherlands. Since Coxella is extremely resistant to heat and desiccation, it can survive in the environment for many months. Outbreaks of this magnitude are unprecedented. Recent European outbreaks in Germany and Switzerland were limited to a short epidemic without significant recurrences in the following years. Up until 2007, an average of 20 cases of human Q fever were reported.
yearly in the Netherlands. The reason for the surge in clinical problems in humans and animals is still unclear. Possible explanations include the increase of the dairy goat population (from 5000 in 1985 to over 375,000 in 2009). Moreover, the type of goat husbandry has changed with sometimes up to thousands of animals in one dairy farm. In contrast to other countries experiencing smaller Q fever epidemics, in the Netherlands these farms are often located in highly populated areas.

After the first year of the epidemic in the Netherlands (with 168 confirmed cases), a limited number of preventive measures were taken. However in 2008, there was a further increase in the number of reported human cases in the Netherlands. Preventive regulations implemented in autumn 2008 consisted of making Q fever a notifiable disease for the veterinary sector (for humans it has been notifiable since 1978), prohibiting the spread of manure from infected farms, and compulsory vaccination for all non-pregnant goats on dairy goat farms in the region. Although vaccination does not eliminate the disease, it is effective in reducing massive bacterial shedding from a heavily infected herd, thereby limiting the risk of environmental contamination. Before 2010, however, there was a considerable shortage of veterinary vaccine and vaccination of all goats was not possible. Therefore, after the further increase in human cases in 2009 (by then with multiple reports of fatal cases, especially of chronic Q fever), drastic measures were taken. Farms were tested by means of polymerase chain reaction testing for Coxiella on bulk milk, and culling of more than 50,000 goats on 88 infected farms was started in December 2009. Furthermore, breeding on those farms was prohibited. Inhabitants of the area within 5 km of the contaminated farms were alerted regarding possible Q fever. A list of positive farms was made available to the public (http://www.vwa.nl/onderwerpen/dierziekten/dossier/q-koorts/kaart-met-overzicht-van-besmette-bedrijven). In 2010, fewer cases of Q fever have been reported compared with 2009. Although differences in weather circumstances could play a role in this decrease, it seems that the measures taken have had a positive effect on limiting transmission to humans.

CLINICAL ASPECTS OF Q FEVER

Acute Q fever
Acute Q fever occurs two to six weeks after exposure depending on the infective dose. The infection remains asymptomatic in up to 60% of patients. Patients with symptomatic disease usually present with fever and mild flu-like symptoms. Because these symptoms are very non-specific, under-reporting is probably quite substantial. A case-control study investigating the first outbreak of Q fever in the Netherlands in 2007 showed that all patients with symptomatic infection experienced fever. Headache and cough were reported by 92 and 85%, respectively. Smoking was found to be an important risk factor, as had been shown by others previously. Male sex has also been identified as a risk factor for symptomatic disease. In 2007 and 2008, the female-to-male ratio of acute Q fever was 1:1.7. The mean age was 51 years. Although hospitalisation rates of 2% have been described in literature, hospital admission was much more frequent in the Netherlands. In 2007, almost 50% of Q fever cases were admitted. This high percentage could have been influenced by active case finding in a retrospective study among hospitalised patients. In the subsequent years, admission rates stabilised to around 20%, still considerably higher than reported in literature.

Chronic Q fever
Chronic Q fever develops in 1 to 2% of patients after acute Q fever. Some patients with chronic Q fever do not recall having had an acute infection. It usually develops insidiously, months or even years after acute infection and patients often present with non-specific symptoms such as low-grade fever, night sweats and weight loss. In a large retrospective study from France, endocarditis was found to be the predominant manifestation of chronic Q fever, constituting 73% of chronic Q fever cases. Other manifestations were vascular infection (8%), chronic infection in pregnancy (6%), and chronic hepatitis (3%). In the Netherlands however, a substantially higher percentage of chronic Q fever cases consists of patients with infected aneurysms and vascular prostheses. In a recent report, 12 out of 22 chronic Q fever patients in the Netherlands had vascular infection. Four of these patients were diagnosed by screening 52 patients with an aortic aneurysm. The authors advocate screening all patients with symptomatic aortic aneurysms in a highly endemic region for chronic Q fever. Chronic Q fever is not systematically reported to the national health authorities in the Netherlands. It is estimated that around 40 to 50 cases of chronic Q fever have been diagnosed in the Netherlands in the past three years. Up to half of these cases have vascular infection. A nationwide database on chronic Q fever will be established to collect these data and facilitate epidemiological research.

Q fever and pregnancy
Literature on chronic Q fever during pregnancy is limited. A case series of 53 pregnant women diagnosed with Q fever showed obstetric complications in 81% of patients not treated with long-term cotrimoxazole therapy compared with 44% in patients who did receive cotrimoxazole. An important pitfall in this observational study, as indicated by the authors, is the fact that serology for Q fever was
Performed only after delivery in 28% of patients, often because of obstetric complications, creating a selection bias. Interpretation of these results is therefore difficult. Two large seroprevalence studies found no significant association between seropositivity for *Coxiella* and adverse pregnancy outcome. A study among pregnant women in the area of the first outbreak in the Netherlands in 2007 showed evidence of recent infection in three out of 19 women (16%). This was significantly higher than in the surrounding regions. A retrospective study in the highly endemic area in the southern part of the Netherlands showed no significant correlation of seropositivity for Q fever during early pregnancy and adverse pregnancy outcome. To further investigate the effect of Q fever on pregnancy, a randomised controlled trial was started in the spring of 2010 evaluating the effect of a screen and treat policy of pregnant women in this area.

**Post Q fever fatigue syndrome**

Following acute Q fever, patients frequently report a long-lasting and debilitating fatigue. A study performed after an outbreak of acute Q fever in the United Kingdom showed 20% of patients suffered from chronic fatigue syndrome after ten years of follow-up, compared with 4% of controls. A study among abattoir employees in Australia showed that 28% of patients with proven acute Q fever still fulfilled the CDC criteria of chronic fatigue syndrome at five years after infection compared with none of seronegative controls. A case-control study among 54 patients who contracted acute Q fever in the first year of the epidemic in the Netherlands showed that after one year, over 50% still reported severe fatigue compared with 26% of controls. The aetiology of this severe fatigue, referred to as QFS (Q fever Fatigue Syndrome), still remains to be elucidated. Cytokine dysregulation resulting from chronic immune stimulation and modulation by persistence of *Coxiella* or its antigens has been hypothesised. Genotyping of patients suffering from QFS showed significant differences in HLA-DRB1*11 and interferon-γ intron 1 microsatellite compared with controls. Some reports suggest persistence of *Coxiella* or antigenic non-viable cell residues in bone marrow. Studies evaluating antibiotic treatment for QFS have shown conflicting results. QFS leads to considerable morbidity and a high socioeconomic burden related to increased use of healthcare facilities and absence from work.

**Diagnosis of Q fever**

Analysis of specimens from various infected dairy farms has shown that 14 different strains circulate in the Netherlands, but one is predominantly present in the highly endemic area (Roest HJ, unpublished data). Isolation of *Coxiella* from blood culture specimens of Q fever patients is difficult since it is an obligatory intracellular organism. In addition, chronic infection often resides in tissues (e.g., heart valves or vascular aneurysms) and shedding into peripheral blood occurs in very low concentrations. Culture of *Coxiella* requires very specific procedures and a biosafety level 3 laboratory, which is not available to most hospitals. Until now, culture of the pathogen has been successful in only one patient in the Netherlands, in whom *Coxiella* was cultured from a resected heart valve (Roest HJ, personal communication). Diagnosis of acute Q fever is based on serology, the reference method being immunofluorescence assay (IFA). A seroconversion of a fourfold rise in antibody titre is diagnostic for acute Q fever. An important drawback in diagnosis based on serology is that antibody production does not usually occur until a few weeks after onset of clinical symptoms. PCR on serum has been shown to have a high sensitivity (98%) for acute Q fever in seronegative patients and is a useful diagnostic tool for early diagnosis. An algorithm for the diagnosis of acute Q fever in the Netherlands, designed by the Dutch working group on diagnostics of acute Q fever (an initiative of the National Institute for Public Health and the Environment (RIVM) and the Dutch Association for Medical Microbiology), has been published very recently. There has been a substantial reduction of the diagnostic delay in the Netherlands from 82 days in 2007 to 20 days in 2009. Because treatment has to be started before laboratory confirmation, physicians have to rely on clinical signs to guide the decision to start empiric therapy for Q fever. Antibiotic treatment of community acquired pneumonia in a high endemic region should include an agent active against *Coxiella burnetii*. Diagnosis of chronic Q fever remains difficult. Because the infection persists intracellularly, PCR on peripheral blood is not always positive. Imaging techniques can be useful to localise infection. Infected aneurysms or vascular prostheses can be identified by FDG positron emission tomography (PET) or CT. In case of endocarditis, however, diagnosis is often more complex. The original Duke criteria developed for diagnosing infective endocarditis include vegetations and positive blood cultures as major criteria. However, vegetations are often absent in Q fever endocarditis and as mentioned above, *Coxiella* does not grow in conventional blood culture media. Therefore a revision of the Duke criteria has been proposed in which a serological profile compatible with chronic Q fever has been added to the major criteria. Serology is therefore an important tool in the evaluation of the development of chronic disease. *Coxiella burnetii* displays a unique antigenic variance in surface polysaccharides (phase 1 and phase 2 antigens). This can be used to distinguish between acute and chronic infection.
In acute infection, mainly phase 2 antibodies develop and convalescent sera show low titres of phase 1 antibodies, whereas chronic infection is characterised by high titres of phase 1 antibodies. Most literature on determination of cut-off values for establishing the diagnosis of chronic Q fever originates from the French National Reference Centre for Rickettsial Diseases (NRC) and this group has proposed a cut-off value for IgG to phase 1 proteins of 1:800 for the diagnosis of chronic Q fever. In the Netherlands, however, a substantial percentage of patients showed much higher titres of IgG1 during the first months after acute infection. There seems to be a considerable difference between the serological method used in the Netherlands (Focus diagnostics) and the method used in the NRC in France. This was recently illustrated by a case report of serological follow-up after acute Q fever, which showed high titres to IgG1 comparable with those found in Dutch patients but much lower when tested in the NRC in France. In 2009, a provisional guideline was published in the Netherlands proposing a new cut-off value for IgG1 of 1:4096 (or an IgG1 equal to IgG2) (www.medischcontact.artsennet.nl). However, when using this algorithm, 40% of patients with proven chronic Q fever (signs and or symptoms compatible with chronic Q fever and persistently positive PCR on blood or positive PCR on resected valves or aneurysms) who presented at the Radboud Expertise Centre for Q fever do not fulfil these criteria (table 1). The Netherlands Society for Medical Microbiology (NVMM) is currently developing a new guideline for the diagnosis of chronic Q fever in the Netherlands.

**TREATMENT OF Q FEVER**

Comparative trials regarding the optimal antibiotic treatment for acute Q fever are sparse, often retrospective and sometimes show conflicting results. Doxycycline is the preferred choice, but the new-generation quinolones such as moxifloxacin are also active against *C. burnetii*. Clarithromycin has also been shown to be effective and co-trimoxazole is the treatment of choice in children younger than 8 years of age. Although the national guidelines for treating community acquired pneumonia (CAP) issued by the Dutch Working Party on Antibiotic Policy (SWAB) and the Dutch College of General Practitioners (NHG) recommend doxycycline for first-line treatment of CAP, the alternative regimens in these guidelines do not routinely cover *C. burnetii*. Nevertheless, most general practitioners in the highly endemic region are aware of this problem and treat their patients with either doxycycline or moxifloxacin. A survey among general practitioners in this region showed that 95% would consider Q fever as a possible pathogen when suspecting a pneumonia and would start empiric treatment with doxycycline. The move away from doxycycline in the proposed update of the NHG guidelines for treatment of pneumonia by general practitioners seems inappropriate for endemic regions and may lead to increased numbers of chronic infections.

The optimal treatment of chronic Q fever consists of doxycycline and hydroxychloroquine. The latter increases the intralysosomal pH and thereby achieves a bactericidal effect in vitro when combined with doxycycline, whereas doxycycline alone is only bacteriostatic. Based on a retrospective study, a minimum duration of 18 months of combination therapy has been recommended with target levels of doxycycline of 5 mg/l. This long-term therapy is associated with significant adverse effects and photosensitisation has been described in 100% of patients on long-term therapy. Other frequently reported side effects include nausea, headache and dizziness. Regular evaluation by an ophthalmologist is recommended because of possible irreversible maculopathy due to hydroxychloroquine. Maintaining optimal adherence to therapy therefore requires intensive counselling and therapeutic drug monitoring.

**Table 1. Clinical and microbiological data from 10 patients with chronic Q fever presented to the Radboud Expertise Centre for Q fever**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Localisation of infection</th>
<th>IgG1 titre at diagnosis</th>
<th>IgG2 titre at diagnosis</th>
<th>PCR Q fever Blood</th>
<th>PCR Q fever Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>M, 75 y</td>
<td>Endocarditis</td>
<td>8192</td>
<td>32768</td>
<td>Positive</td>
<td>NA</td>
</tr>
<tr>
<td>M, 62 y</td>
<td>Aneurysm</td>
<td>2048</td>
<td>4096</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>M, 69 y</td>
<td>Endocarditis</td>
<td>65536</td>
<td>65536</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>M, 69 y</td>
<td>Endocarditis</td>
<td>16384</td>
<td>16384</td>
<td>Positive</td>
<td>NA</td>
</tr>
<tr>
<td>M, 57 y</td>
<td>Aneurysm</td>
<td>1024</td>
<td>1024</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>M, 68 y</td>
<td>Aneurysm</td>
<td>4096</td>
<td>4096</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>M, 75 y</td>
<td>Endocarditis</td>
<td>&gt;4096</td>
<td>&gt;4096</td>
<td>Positive</td>
<td>NA</td>
</tr>
<tr>
<td>M, 62 y</td>
<td>Aneurysm</td>
<td>1024</td>
<td>&gt;4096</td>
<td>Positive</td>
<td>NA</td>
</tr>
<tr>
<td>M, 67 y</td>
<td>Aneurysm</td>
<td>2048</td>
<td>2048</td>
<td>Positive</td>
<td>NA</td>
</tr>
<tr>
<td>F, 60 y</td>
<td>Unknown</td>
<td>8192</td>
<td>4096</td>
<td>Negative</td>
<td>NA</td>
</tr>
</tbody>
</table>
PREVENTION OF Q FEVER IN HUMANS

The preventive measures taken in the veterinary sector have been aimed at reducing the spread of *C. burnetii* in the environment and thereby limiting the transmission to humans. Human vaccination is a different approach in preventing Q fever in individuals with a high risk of exposure to *Coxiella*. An effective whole-cell vaccine is available in Australia and has been extensively used in persons with high occupational risks such as abattoir employees. In this population, it has been proven to be highly effective.\(^{49}\) Because administering this vaccine to patients with pre-existing immunity increases the risk of local and systemic inflammatory reactions, prior infection needs to be excluded by skin testing and serology. Recently, the Health Council of the Netherlands issued an advice on vaccinating patients with increased risk of chronic Q fever with this whole cell vaccine (http://www.gezondheidsraad.nl/nl/adviezen/vaccinatie-van-mensen-egen-kooarts-eerste-advies). The target population has been defined as patients with underlying cardiac conditions (the same category of patients who would also qualify for endocarditis prophylaxis according to current guidelines), as well as patients with a known (aortic) aneurysm or vascular prosthesis.

Since Q fever is highly endemic in the southern part of the Netherlands and infection can be asymptomatic, there is a possible risk of transmission through blood transfusion. Preliminary results indicate that in 2009 *C. burnetii* DNA was present in a small percentage of blood donations in this area (indicated by positive PCR on donated blood).\(^{15,50}\) Sanquin, the Dutch blood supply foundation, has instituted screening of donated blood in the high-incidence area as a precautionary measure.

CONCLUSION

Although it appears that the epidemic of Q fever in the Netherlands is now subsiding, physicians are still faced with growing numbers of patients suffering from long-term sequelae of Q fever such as chronic infection and Q fever fatigue syndrome. Optimal management of these conditions is still unclear and further research is needed to improve diagnostic strategies, to evaluate treatment, and to prevent chronic infections.

REFERENCES


Non-invasive measurements of atherosclerosis (NIMA): current evidence and future perspectives

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ABSTRACT

In clinical practice, cardiovascular (CV) risk stratification is based on the assessment of individual risk factors. Still many cardiovascular deaths occur in individuals who were not at high risk according to the current CV risk stratification models as the Systematic Coronary Risk Evaluation chart (SCORE) and Framingham Risk Score. By measuring morphological and/or functional abnormalities in the arterial wall directly, the impact of all CV risk factors together can be determined. In this review, the current status for the use of a panel of non-invasive measurements of atherosclerosis (NIMA) in CV risk prediction in clinical practice is discussed. Some of these NIMA showed predictive value for CV disease, such as intima-media thickness, pulse wave velocity, and ankle-brachial index, both in patients and in healthy and community-based populations. Recommendations have been made to include these NIMA in CV risk stratification in secondary prevention. However, the additional value of NIMA in CV risk stratification in primary prevention settings remains to be determined. Furthermore, the main determinants of NIMA are still unclear. Also the use of different combinations of NIMA should be evaluated, since different NIMA likely reflect different stages and aspects of the atherosclerotic process that leads to CV events. Future prospective studies should focus on repeated measures of NIMA to reveal the main determinants of the different NIMA and evaluate the predictive value of baseline versus repeated measurements.

KEYWORDS

Imaging, atherosclerosis, arterial stiffness, intima-media-thickness, ankle-brachial index

INTRODUCTION

Cardiovascular risk
Cardiovascular disease (CVD) has been a major cause of death for decades now, and it will probably be for years hereafter, although the number of cardiovascular deaths is decreasing. In 2005, 17.5 million cardiovascular deaths were registered, which was 30% of all global deaths and in the Netherlands a comparable trend was observed. Atherosclerosis is the major underlying process, leading to cardiovascular events. Many risk factors have been identified that promote atherosclerosis, including obesity, hypertension, lipid disorders, smoking, and diabetes mellitus. Cardiovascular (CV) risk prediction is mainly based on the assessment of these individual CV risk factors. Often only the most conventional CV risk factors are determined and treated to reduce CV risk. We do not know why some individuals develop early CVD and others do not, despite the presence of risk factors. Many CV deaths occur in patients who were not identified as high-risk patients. Moreover, despite blood pressure control, optimising lipid levels and lifestyle advice, approximately 50% of the patients who died from cardiac arrest were in the intermediate risk category of Framingham Risk Score, as described by Taylor in 2002. Therefore, in the last few years research has focused on new biomarkers of atherosclerosis, including markers of inflammation and oxidative stress. So far, none of the new biomarkers appeared to have additional prognostic power in CV risk prediction beyond the traditional risk scores. At every level of traditional risk factor exposure, there is a large inter-individual variation in the amount of atherosclerosis and the development of CVD. This variation is probably due to genetic susceptibility, combinations and interactions between risk factors,
including lifestyle habits, duration of exposure to specific levels of the risk factors, and factors such as biological and laboratory variability. When patients present at the clinic with a CV event, most of the damage has already been done. Therefore, atherosclerosis must be discovered as early as possible in primary prevention settings.

**Non-invasive measurements of atherosclerosis (NIMA)**

A current concept is that by measuring atherosclerosis directly in the arterial wall, the damage caused by known and unknown risk factors can be determined, which allows us to better predict CV risk for the individual patient. This also provides the opportunity to measure atherosclerosis before clinically overt CVD, as changes in the arterial wall precede the clinical symptoms of CVD. Thus, subclinical disease measurements, representing the final result of risk exposure and genetic susceptibility, may be useful for improving CVD risk stratification, therapeutic strategies and evaluation of risk factor modification. Several invasive techniques to visualise the arterial system and the extent of atherosclerosis are available, such as intravascular ultrasound and angiography; the latter has been the ‘gold standard’ imaging technique for the presence of stenosis and/or occlusions in the arterial system in clinical practice for years now. It does not need further explanation that invasive techniques are not suitable as a screening tool in the general population. More recently, less invasive techniques became available, such as computed tomography and magnetic resonance imaging, although sometimes detergents are needed to optimise the pictures, which have to be injected. Moreover, these techniques are not widely available and very expensive at the moment, they cannot be applied to every patient, and expose patients to radiation. Therefore, many efforts have been made to develop relatively simple and cheap non-invasive measurements of atherosclerosis that can be applied to every individual. A variety of these non-invasive measurements have been developed in the last few years, each measuring different aspects of the atherosclerotic process. In this review, we explore the use of a panel of these non-invasive measurements and derived parameters, as depicted in figure 1. The NIMA will be discussed in the sections below based on our own experience, including their current status and evidence for introduction of these NIMA into clinical practice in primary prevention. We will conclude with future perspectives of NIMA in relation to cardiovascular risk prediction.

**ENDOTHELIAL (DYS)FUNCTION**

**Flow-mediated dilation**

Dysfunction of the endothelium, a monolayer of cells that covers the intima, is one of the first signs of atherosclerosis and is present before structural changes. Endothelial (dys)function can be measured non-invasively by flow-mediated dilation (FMD) with ultrasound at the brachial artery (figure 2). It has been recommended to perform FMD measurements according to the guidelines of the International Brachial Artery Reactivity Task Force. In short: changes in the diameter of the brachial artery are measured at baseline and after releasing a cuff that has occluded the artery for four minutes. This results in an increased blood flow to restore the circulation,

![Figure 1. Cross-section of an artery with progressive atherosclerotic lesions including the different non-invasive measurements of vascular abnormalities and the derived parameters. In the boxes at the bottom, the change in the NIMA parameters with progression of atherosclerosis is depicted.](image-url)
leading to increased shear stress on the endothelium. A healthy endothelium produces nitric oxide (NO), which causes dilation of the artery to increase the blood flow to the peripheral circulation. When there is endothelial dysfunction, less NO is produced leading to less dilation of the artery. FMD is calculated as the post-occlusion diameter divided by the baseline diameter and is expressed as a percentage. When endothelial function is impaired, a lower FMD is measured.

An important limitation of FMD is its relatively large variability. Numerous factors, such as biological and technical factors, contribute to the variability of FMD as recently summarised by Moens and co-workers. Many efforts have been made to reduce measurement variability, such as the introduction of monitoring software. Intra-observer coefficients of variation of 1.8 to 23.0% were previously reported, but when expressed as a percentage, coefficients of variation increased to 28 to 33%. To detect a clinical treatment benefit, a mean improvement of FMD of over 2% is necessary and to account for natural variability even a difference of 4 to 8% is necessary. A power analysis showed us that to detect a difference of 0.5% in the prevalence of CVD, over 14,000 FMD measurements are needed. Furthermore, it is a time-consuming measurement that is relatively uncomfortable for the patient. Finally, there has been an under-reporting of negative studies. Reference values for FMD are lacking and depend on the method used; some report FMD after upper arm occlusion whereas others use forearm occlusion.

Despite the relatively large variability, FMD seems to be a promising technique for cardiovascular risk assessment in selected high-risk patient groups and several papers have reported the usefulness of FMD as a tool in CV risk stratification and prediction, although prospective studies are needed to prove this concept. Until recently, prospective data of population-based cohorts were scarce and the reported results were not consistent; FMD was related to CV events in some, but not all studies in the general population. These inconsistencies might be the result of the reported variability. Previously, we reported that FMD was not related to the traditional CV risk factors or prevalent CVD, neither in a low-risk nor a high-risk population including patients with familial combined hyperlipidaemia (FCH). Endothelial dysfunction is a measure of early atherosclerosis and will therefore be present in older populations where multiple risk factors have been present for many years. Very recently, Yeboah and colleagues showed that FMD was a predictor of CVD in a large sample from the general population, although FMD did not improve the prediction of CVD over the Framingham Risk Score. However, adding FMD to risk stratification based on the Framingham Risk Score made many individuals with a normal FMD shift towards a lower risk category. They concluded that FMD might help in CV risk stratification to select those who seemed to be at risk based on Framingham Risk Score, but based on a normal FMD seem to be at lower CV risk. These conclusions have to be regarded with care because of the variability of FMD, as also reported by these authors.

After all these years of research on FMD, there is still no clarity on its possible potential to be a screening tool in CV risk stratification and no uniform results have been reported. Therefore, the use of FMD in clinical practice and especially in primary prevention settings is questionable and the time for FMD to be applied in clinical practice is still far away. Further research should focus on the possible role of FMD in CV risk stratification in younger populations, using standardised methods for FMD in standardised conditions. Improved or other non-invasive measures of endothelial function have to be developed and investigated for their applicability in clinical practice.
Nitroglycerin-mediated dilation

Beside endothelium-dependent vasodilation, the endothelial-independent vasodilation can be determined by administration of nitroglycerin. Nitroglycerin causes relaxation of the smooth muscle cells, which results in dilation of the arteries, and is independent of the function of the endothelium. The maximum diameter after nitroglycerin is divided by the baseline diameter and expressed as a percentage. When the function of the smooth muscle cells is impaired, the nitroglycerin-mediated dilation (NMD) is decreased. The NMD is used to check whether the attenuation of FMD is caused by damage in the endothelium and not a consequence of changes in the smooth muscle cells. Reference values have never been reported and, just as with FMD, depend on the method used. The role of NMD in CV risk stratification is unclear and most likely limited.

Brachial artery diameter

The brachial artery diameter (BAD) is the measure on which FMD is based. The reported measurement variability is much smaller than for FMD.34 Reference values are lacking, BAD differs between men and women, is dependent on blood pressure, and is influenced by antihypertensive medications. BAD appeared to have predictive value in CV risk assessment in recent publications.30,35,36 We previously reported that BAD was related to cardiovascular risk factors and other measurements of subclinical atherosclerosis in our population-based sample.18 The diameter of the brachial artery might be a reflection of systemic vasodilation, as a compensation in reaction to narrowing of the arterial lumen.37 The BAD might be a potential tool in CV risk stratification when combined with other measurements of atherosclerosis; however, this has to be evaluated prospectively.

ARTERIAL STIFFNESS

Due to ageing and the progression of atherosclerosis, the arterial wall changes, and besides dysfunction of the endothelium, these changes result in arterial stiffness.38 Arterial stiffness can be measured non-invasively with pulse wave analysis (PWA) and pulse wave velocity (PWV), using tonometry, as depicted in figure 3. The heart ejects a bolus of blood into the arterial system with every heartbeat and this causes a blood pressure wave through the arteries. When the wave arrives at an artery, this causes expansion of the artery. This phenomenon can be observed as the arterial pulse, which can be palpated at the wrist or at the carotid artery. A tonometer is a device that registers the changes in diameter of arteries.

Due to the different composition of central and peripheral arteries, not all arteries stiffen to the same extent. The stiffening of central arteries is greater than the stiffening of the peripheral arteries. The clinical consequences of arterial stiffness are an increased risk of stroke as a result of increased systolic blood pressure, the development of left ventricular hypertrophy as a result of increased cardiac after load, and a decrease in coronary perfusion and heart failure due to the decrease in diastolic blood pressure.

Pulse wave velocity (PWV)

PWV is a measure of wave velocity, which is propagated by contraction of the heart and travels along the arterial tree. To determine PWV, pulse waveforms are recorded at two sites sequentially, and wave transit time can be calculated using the R wave of a simultaneously recorded electrocardiography as a reference frame. PWV measured between the right carotid and the left femoral artery has been described as the gold standard measurement of arterial stiffness.
arterial stiffness by a panel of experts. Surface distance between the two recording sites can be measured parallel to the plane of the examination table. The distance between the carotid artery site and the supra sternal notch has to be subtracted from the distance between the supra sternal notch and the femoral artery site. PWV is calculated by dividing the travelled distance by the time. As the arteries become stiffer with ageing and progression of atherosclerosis, PWV increases. To minimise variability and make comparison between studies possible, recommendations for user procedures were provided by Van Bortel et al. Reproducibility of PWV has been extensively studied and measurement variability is rather small. Reference values have been provided by several authors in healthy adults the PWV generally ranges from 6 to 11 m/sec. The guidelines for the management of arterial hypertension and the guidelines for cardiovascular screening in the asymptomatic at-risk population included a PWV value >12 m/s as a sign of target organ damage.

PWV is an independent predictor of CVD in selected high-risk patient groups, and in the general population and could provide additional information in clinical practice for CV risk stratification. Very recently, two studies even reported an improvement of CV risk stratification by adding PWV in hypertensive patients. The problem of these derived parameters is the calculation by means of a transfer function, which has only been validated in selected patient groups. Therefore, care must be taken when interpreting the data in other populations. Furthermore, there is doubt about the formula used to calculate the AIx. Since AIx strongly depends on heart rate, AIx corrected for a heart rate of 75 beats per minute is used. Different techniques are used to measure AIx and not all provide central pressure parameters. Reported reproducibility of the different techniques is good. Also recently, reference values were reported in different populations. As atherosclerosis increases, AIx increases and this increase has been associated with increased CV risk. In FCH patients we could not report a difference in AIx compared with their unaffected relatives, whereas PWV was increased. This discrepancy might be explained by the fact that the age-related changes in PWV and AIx follow different patterns; changes in AIx are more dominant in younger subjects (<50 years) and changes in PWV are more marked in older individuals (>50 years). In a population-based cohort we found that AIx was associated with the metabolic syndrome and its individual traits, although the association was stronger in men than in women. AIx also modestly but significantly increased with increasing apoB levels, but not with increasing waist circumference.

The use of central blood pressure parameters recently regained interest due to the results of the Conduit Artery Functional Endpoint (CAFÉ) study showing that central blood pressure but not peripheral blood pressure was lowered by one of the drugs administered. Since then, many studies have incorporated the central pressure measurements and many results have to be awaited. Very recently the same authors published additional analyses and concluded that the difference in central pressure reported before was mainly the result of the heart rate reduction with β-blockers. This appeared to be the major mechanism accounting for less effective central aortic pressure reduction per unit change in brachial pressure. Also the Cardiovascular Health Study showed that central pressure was more strongly related to (subclinical) atherosclerosis and CV events than brachial blood pressure, which was strengthened by a
review in 2009.7 Very recently, a systematic review and meta-analysis provided quite robust evidence that central haemodynamics are independent predictors of CV events and all-cause mortality in different patient groups.8 To summarise, evidence is accumulating that central pressures would be more useful in CV risk stratification, although the independent predictive value of central haemodynamics in primary prevention remains to be determined.

THICKNESS OF THE ARTERIAL WALL

Intima-media thickness (IMT)
The arterial wall can be visualised and the thickness of the arterial wall can be measured using ultrasound, as depicted in figure 4.

IMT measures structural changes in the arterial wall and is a well-established marker of (subclinical) atherosclerosis. With ageing and progression of atherosclerosis the arterial wall thickens. The increase in IMT is associated with unfavourable levels of cardiovascular risk factors, atherosclerosis elsewhere in the arterial system, and with cardiovascular disease.78-83 IMT can be measured at different sites of the arterial tree. The most common place to measure IMT is the distal common carotid artery. The presence of a plaque is defined by the Mannheim intima-media thickness consensus as a focal thickening of the arterial wall of at least 1.5 x the mean IMT.84 Numerous studies have reported that IMT can be measured in a reliable and reproducible manner, although different methods and definitions are used in different studies.85,86 Measurement variability is typically introduced from several resources: ultrasound scanning equipment, sonographers, reading equipment, readers of the scans, scanning protocol, and thickness of the intima-media complex. Since automatic devices were introduced to measure IMT, variability decreased substantially.87-90 At higher ages, the variability in IMT between subjects is larger.91 The thickness of the wall also varies during the heart cycle. In the diastolic phase, the IMT is thicker than in the systolic phase. Therefore the measurements have to be performed at the same phase of the heart cycle in every person. Several authors have already extensively discussed the different methods used to measure IMT and international recommendations have been made for standardised IMT measurements.92 In studies evaluating the effect of drugs in which IMT is the primary endpoint, very small differences have to be detected, demanding a very precise IMT measurement. These studies mostly include IMT measures of the common carotid artery, the bulbus and the internal carotid artery measured from different scanning angles. Other studies use IMT as a screening tool; the measurement then needs to be simple, quick, but reliable and most studies only measure the IMT of the common carotid arteries at the angle that showed the optical thickest IMT.

Reference values were provided by many studies stratified by age.86,99-96 The guidelines for the management of arterial hypertension and the guidelines for cardiovascular screening in the asymptomatic at-risk population included an IMT value >0.9 mm or the presence of plaque as a sign of target organ damage.46-47 IMT has shown to predict CVD, in patients as well as in asymptomatic individuals.80-97,103 In line with these data, we showed that IMT was strongly associated with traditional CV risk factors in both participants from a population based sample,58,59 and in a high-risk population.104 The additive value of IMT over and above traditional CV risk factors in CV risk stratification has not been proven yet.105-107 Only one relatively small study reported that IMT would improve CV risk stratification in a primary prevention setting.108 In summary, IMT is a well-established surrogate marker of atherosclerosis and is a very promising tool to be included in CV risk stratification in clinical practice in the near future. The additive value in primary prevention remains to be determined prospectively.

The presence of plaque and plaque thickness
The presence of plaque and plaque thickness are measures of advanced atherosclerosis. Not many studies have included these parameters, and those that did used many different methods and definitions. As described in the previous section, the presence of plaque showed predictive value for CVD and is included in some guidelines.47 Recommendations have been reported on how to define the presence of plaque.44 We reported in our low-risk population that participants with the metabolic syndrome...
had thicker plaques than those without. The number of participants with plaques present increased with increasing apoB levels and increasing waist circumference. The predictive value of plaque thickness in CV risk stratification and the additive value of the presence of plaque in different populations need to be evaluated in prospective studies, taking into account the measuring method.

P E R I P H E R A L  F L O W

Ankle-brachial index at rest
The ankle-brachial index (ABI) at rest measures more advanced stages of atherosclerosis and has been used in clinical practice for years now to determine whether a patient suffers from peripheral arterial disease. The method commonly applied is one measurement of ABI at a single time point and by one single observer based on the publication of Price et al, who established the cut-off value of ABI in a very large population-based cohort. The measurement found its way into clinical practice rather easily. The first publication on the reproducibility of the ABI dates from 1981. The authors recommended performing the measurement more than once and a difference in subsequent measures from 15 mmHg could be regarded as clinically relevant. Reproducibility of the ABI has been studied in selected patient groups and was reliable when performed by trained technicians. An ABI at rest below 0.9 is widely considered to be abnormal. The ABI at rest is a simple, non-invasive and inexpensive test that can be used to identify individuals who are at high risk of developing CVD. Several studies have reported that a low ABI at rest had predictive value for CVD in patients with CVD and in low-risk populations. We reported that a decreased ABI at rest was associated with the metabolic syndrome and its individual traits in our population-based cohort. A decreased ABI was also observed with increasing waist and its individual traits in our population-based cohort.58 ABI at rest was associated with the metabolic syndrome had a decreased ABI after exercise in the general population. ABI after exercise also decreased with increasing waist circumference and with increasing apoB levels. Further studies are warranted to determine the predictive value of ABI after exercise for CVD and its additive value over and beyond traditional CV risk factors, especially in low-risk populations in primary prevention settings.

To summarise: although most of the NIMA described in this review are used for research purposes worldwide, none of these measurements have made their way into clinical practice yet, except for the ankle-brachial index (ABI). Some NIMA, including ABI, IMT, and PWV, have been recommended to be included in cardiovascular risk stratification to determine subclinical organ damage in the guidelines for the management of arterial hypertension, and in the guidelines for CV screening in the asymptomatic at-risk population.

P R E D I C T I V E  V A L U E  O F  C O M B I N A T I O N S  O F  N I M A

Each NIMA reflects a different characteristic of the atherosclerotic process, involving functional and/or morphological changes in the vessel wall. It might be better to define the measurements as non-invasive measurements of vascular abnormalities (NIMVA) than NIMA. It is also known that the extent of atherosclerosis differs along the arterial tree. In different populations at risk of CVD, different characteristics of the atherosclerotic process may be present or accelerated. Therefore, simultaneous measurements of different NIMVA could theoretically enhance the power to improve CV risk stratification. Only very few studies have evaluated the predictive value for CVD for different combinations of NIMVA. Very recently, Novo and co-workers reported that IMT in combination with the presence of plaque might provide additional information on CV risk in a primary prevention setting. This was also recently reported in the Atherosclerosis Risk In Communities (ARIC) study by Nambi et al. Tu and co-workers reported on the predictive value of the combination of IMT and arterial stiffness, but not all measures of stiffness used in that study showed the same results. In contrast, Muiesan and colleagues found that PWV in combination with echocardiography enhanced CV risk stratification, but adding PWV to IMT did not.
Further studies need to investigate which NIMVA should best be combined to improve CV risk stratification, as well in primary as in secondary prevention.

Determinants of NIMVA

The progression rate of atherosclerosis differs among individuals. This might be due to different impact of known and unknown CV risk factors and/or differences in time exposure to these risk factors, and/or genetic predisposition. NIMVA measure the extent of vascular abnormalities reflecting (subclinical) atherosclerosis and the hypothesis is that this amount of atherosclerosis reflects the impact of all different CV risk factors together. Previous studies demonstrated that the predictive power of some individual NIMVA for cardiovascular events is independent of the conventional risk factors as described in the previous sections. Still a large proportion of the variance in NIMVA remained unexplained. In general, the reported percentage explained variance in NIMVA is larger in high-risk populations (i.e. ±50%) than in low-risk populations (i.e. ±30%).125-131 In line with these data we reported the percentage explained variance in IMT of ±50%, in both FCH patients and in the unaffected relatives.104 Future studies are needed to identify other main determinants of NIMVA, including exploring potential new CV risk factors. Repeated measurements of NIMVA might help to unravel the impact of ageing, time of exposure to known and unknown CV risk factors, and/or genetic susceptibility.

Future Perspectives

Subclinical disease measurements i.e. NIMVA may be useful for improving CV risk prediction, therapeutic strategies and evaluation of risk factor modification. However, the major pathophysiological determinants of NIMVA are still unknown. Reference values for primary prevention are still lacking for most of the described NIMVA. Furthermore, follow-up data on the panel of NIMVA are not yet available and therefore the relevance of NIMVA in clinical practice for the individual patient is unclear. Measuring changes in a panel of NIMVA values after, for instance, five years of follow-up, in both a low- and a high-risk population, in relation to changes in traditional and new CV risk factors and incidence of CVD, will unravel the major pathophysiological determinants of NIMVA, including ageing, time of risk factor exposure, and genetic risk factors. Also the power of baseline versus repeated NIMVA in CVD risk prediction, over and beyond CV risk factors, can be determined, leading to an evidence-based protocol for NIMVA to improve cardiovascular risk stratification for the individual patient in clinical practice. Furthermore, the combination of NIMVA that will improve CV risk stratification in both low- and high-risk populations in a cost-effective way can be unravelled, allowing earlier and more effective (new) preventive therapy.

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References


Time for a comeback of NSAIDs in proteinuric chronic kidney disease?

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ABSTRACT

Before the introduction of renin-angiotensin-aldosterone system (RAAS) inhibitors in the 1980s, non-steroidal anti-inflammatory drugs (NSAIDs) were the only class of drugs available for the reduction of symptomatic proteinuria. Long-term data from those days suggested sustained renoprotective properties in proteinuric chronic kidney disease (CKD), but this potential has not been further explored, due to the adverse effects of NSAIDs, and due to the successful introduction of RAAS blockade for blood pressure control and renoprotection. The renoprotective potential of NSAIDs may seem surprising for the present generation of clinicians, as NSAIDs are well known for their adverse effects on the kidney. Interestingly, the newer selective COX-2 inhibitors (coxibs), such as non-selective (ns) NSAIDs, exert an antiproteinuric effect in CKD patients. This review discusses the role of NSAIDs as a class of drugs representing an old concept for renoprotection in the light of current insights on renoprotection. It has become increasingly clear during the last two decades, from evidence obtained almost exclusively in studies using RAAS blockade, that not only reduction of blood pressure, but also of proteinuria is a prerequisite for long-term renoprotection. Ns-NSAIDs and coxibs reduce proteinuria without reduction of blood pressure. Their possible role as an adjunct in individualised treatment strategies, particularly for individual patients resistant or intolerant to current therapy, will be discussed.

KEYWORDS

Proteinuria, nephrotic syndrome, kidney disease, NSAID, renin-angiotensin-aldosterone system, COX-2 inhibitors, prostaglandins

INTRODUCTION

Much of our current understanding of the mechanism by which drugs exert protection against progressive renal function decline is derived from randomised controlled trials (RCTs) comparing traditional antihypertensive agents with renin-angiotensin-aldosterone (RAAS) blockade in different renal populations. Reduction of blood pressure has long been recognised as a cornerstone in the treatment of chronic kidney disease, being an important prerequisite to protect against progressive renal function loss as well as against cardiovascular complications. RAAS blockade turned out to be particularly effective to that purpose. Interestingly, the extent of renoprotection exerted by RAAS blockade was larger than could be explained by blood pressure lowering alone, pointing towards specific renal protection. Reduction of glomerular pressure was assumed to be important in this respect, alleviating hypertensive glomerular capillary damage and hence glomerular protein leakage. Interestingly, when more data from RCTs became available, it turned out that the available data consistently showed better renoprotection in the treatment arm with the best proteinuria reduction (usually the RAAS blockade arm), and also, within treatment groups on a specific regimen better renoprotection was seen in individuals with more effective proteinuria reduction. This was in line with the increasing body of evidence showing, first, that proteinuria is a main predictor of renal function loss, and second, that leaked proteins are an important pathophysiological trigger for renal tubulo-interstitial damage. Moreover, it became increasingly clear that proteinuria is a major risk factor for cardiovascular events. So, the ample evidence over the last three decades indicates that, in addition to adequate blood pressure control, proteinuria reduction should be an independent treatment target for renoprotection.
Current guidelines, therefore, recommend not only strict blood pressure lowering (<125/<75 mmHg) for proteinuric patients, but also reduction of proteinuria to <1 g/day, and it has been argued that an even lower target (<0.3 g/day) should be pursued. 3,11 RAAS blockade is the cornerstone in this symptomatic approach. In specific glomerulopathies, such as idiopathic focal and segmental glomerular sclerosis, IgA nephropathy or membranous glomerulopathy, remission of proteinuria and renoprotection may preferably be induced by immunosuppressants, as reviewed previously. 12 Nevertheless, many patients depend on symptomatic therapy because immunosuppressive therapy is either ineffective or causes too many side effects. Before focussing on the role of NSAIDs in the symptomatic treatment of proteinuric CKD patients, the progression that has been made to improve current treatment schedules will be discussed in short.

**INDIVIDUALISED MULTIFACTORIAL APPROACH**

Despite proven renoprotective efficacy of RAAS blockade, the residual renal and cardiovascular risk of treated patients remains very high. For example, in the RENAAL study, conducted in type 2 diabetic nephropathy, the development of ESRD was delayed by approximately 11 months only by the losartan treatment, and the event rate, albeit reduced by some 30%, was still considerably above that in the general population. 13 To improve outcome, therefore, individualised titration regimens have been advocated to obtain control of blood pressure and proteinuria at values recommended by current guidelines. Different stepped-care ‘remission regimens’ were tested comprising dose titration with a single RAAS blocker, dual RAAS blockade (ACE inhibitor plus AT1 receptor blocker (ARB)), enhancement of therapy effect by correction of extracellular volume overload (dietary sodium restriction and/or diuretic use), addition of a calcium antagonist, and lipid control.14-16 Ruggenenti et al. demonstrated the efficacy of such a ‘remission regimen’, showing a much slower decline of eGFR as compared with a matched historical reference group originating from the REIN study treated with monotherapy ramipril in non-diabetic proteinuric glomerulopathy.15 However, the feasibility of this strategy is limited, as many patients do not reach the treatment targets, either due to adverse events (e.g. hyperkalaemia, renal function impairment and hypotension) that preclude maximal titration, or an incomplete response despite maximal titration.15,16 Moreover, there is some evidence that

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**Figure 1. A. Substrates of NSAIDs: cyclooxygenase-1 (COX-1) and COX-2. B. Simplified reproduction of the renal haemodynamic effects of prostaglandins (PG), including PGE2, and angiotensin II (AngII). The blocking effects of NSAIDs and ACE inhibitors (ACEi) on PG and AngII, respectively, will lead to reduction of glomerular pressure and lower urinary protein excretion. PGs affect GFR and ERPF in parallel, whereas AngII has opposite effects to GFR and ERPF, leading to alteration of FF.**

GFR = glomerular filtration rate; ERPF = effective renal plasma flow; FF = filtration fraction (GFR / ERPF).
aggressive down-titration of blood pressure to levels below a systolic of 110 mmHg may be associated with a worse long-term renal outcome. These data underscore that drugs with antiproteinuric properties by a non-hypotensive mechanism deserve exploration. Furthermore, the residual renal and cardiovascular risk reflected by inadequately lowered proteinuria constitutes an unmet need, demanding additive treatment strategies.

**NSAIDS IN A HISTORIC PERSPECTIVE**

From the mid-1950s until the mid-1980s, the potency of non-selective (ns) NSAIDs in reducing proteinuria was tested. Amongst others, Arisz and Donker introduced the ns-NSAID indomethacin to reduce proteinuria in steroid-resistant nephrotic syndrome. In those days, the renoprotective effect of proteinuria reduction was not yet known, but proteinuria was considered a target for treating nephrotic symptoms such as oedema and low serum albumin with consequent catabolic state. To improve the latter, patients generally received liberal protein diets until, in the mid-1980s, it was discovered that a low protein diet improved proteinuria and serum albumin levels. Thus, the clinical context of the early proteinuria reduction studies was quite different from today. In those early studies in heavily proteinuric patients, indomethacin, combined with low sodium diet and hydrochlorothiazide, effectively reduced proteinuria without affecting blood pressure. The antiproteinuric effect was strongly modified by the state of sodium balance, ranging from -80% during sodium depletion to zero effect during volume overload. The reduction in proteinuria during NSAID treatment was accompanied by a proportional reduction in effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) that was more pronounced during sodium depletion as well. Generally, these renal effects are attributed to inhibition of renal prostaglandin production, as supported by the close correlation between reduction in urinary prostaglandin E2 (PGE2) excretion and the antiproteinuric effects of four different NSAIDs, indomethacin 150 mg being the most potent, followed by diclofenac and flurbiprofen, whereas sulindac had hardly any effect. Prostaglandins are known to affect the kidney by modulating vascular tone, glomerular filtration, salt and water homeostasis and renin secretion. In the injured kidney, inducible cyclooxygenase 2 (COX-2) is upregulated and newly expressed in the renal tissue (uniquely the macula densa and adjacent cortical thick ascending limbs) and accounts for the main part of PGE2 production (figure 1). In the previous studies, the effects of indomethacin on ERPF, GFR, and plasma renin activity were also closely associated with the inhibitory effect on PGE2 (figure 2). Consequently, the antiproteinuric response to indomethacin has largely been attributed to these haemodynamic effects reflecting the reduction of intraglomerular pressure by predominantly afferent vasoconstriction leading to reduced glomerular leakage of proteins. Also, in diabetic nephropathy it has been demonstrated that indomethacin 150 mg reduces albuminuria up to 70%. As in non-diabetic proteinuria, proteinuria reduction by indomethacin is accompanied by a reduction in urinary PGE2 excretion and GFR. Given its high antiproteinuric efficacy in different patients, highly dosed indomethacin (150 mg daily) passed for the ‘gold standard’ of symptomatic proteinuria reduction, even in the early years after introduction of the first ACE inhibitor captopril. Data from a more recent head-to-head-comparison study show that both the ACE inhibitor lisinopril (40 mg/day) and ARB (candesartan 32 mg/
day) at maximum recommended doses are more or less equipotent to indomethacin, whereas the COX-2 inhibitor rofecoxib was somewhat less potent (figure 3A). Of note, the combination of NSAIDs and RAAS blockade has been tested as well. Indomethacin has added effects on top of adequately dosed RAAS blockade (figure 3B), indicating that these two modes of intervention act by a different mechanism. As regards renal haemodynamics, indomethacin induces preglomerular vasoconstriction, whereas lisinopril induces postglomerular vasodilation: thus their combination leads to added reduction of glomerular filtration pressure and hence GFR and glomerular protein leakage.

Only two retrospective studies are available with data on long-term outcome with NSAIDs. The uncontrolled study by Lagrue et al. (1988) compared outcomes in patients treated with ns-NSAIDs with outcomes in four different series of patients not treated with ns-NSAIDs. Patients on NSAID developed ESRD in approximately 15 vs 50% at ten-year follow-up. Another retrospective study published in the same period showed similar results in 98 nephrotic-range proteinuric patients with rather preserved renal function (defined as serum creatinine <110 µmol/l) at baseline. At ten-year follow-up, 31% of the patients treated with indomethacin vs 66% not treated with an antiproteinuric drug became dialysis dependent. In this study, no perfect match for baseline characteristics was established, having patients on indomethacin treatment with significantly higher proteinuria, lower blood pressure, and better preserved renal function, as compared with the control group. The suggested long-term protective effects of ns-NSAIDs on renal function have never been tested in a controlled prospective manner, however, due to frequent non-renal as well as renal adverse effects of NSAIDs. Highly dosed indomethacin not only placed the patients at risk for gastrointestinal bleeding, but many patients also did not tolerate these doses because of adverse effects on the central nervous system, e.g. non-orthostatic dizziness and somnolence. Another unwanted effect of NSAIDs comprises water and sodium retention, elevation of blood pressure and development of oedema, potentially annulling the renoprotective effects of proteinuria reduction. Finally, in patients with advanced renal function impairment, the use of NSAIDs is hampered by the decrease in GFR that accompanies an effective proteinuria reduction, as well as the propensity to hyperkalaemia due to specific tubular effects.

**Platelet-aggregating inhibitory agents**

Platelet-aggregating inhibitory agents may exert renoprotective effects as well, although they may not strictly be classified as NSAIDs. Nevertheless, these agents exert their effects by inhibiting prostaglandin synthesis for a great part, particularly by inhibition of COX-1 (figure 1). Platelet-aggregating inhibitory agents, firstly, affect platelet activity, thereby preventing endothelial dysfunction, microangiopathy and accelerated atherosclerosis, and microalbuminuria. Secondly, platelet-aggregating inhibitory agents also exert their effects of ns-NSAIDs on renal function have never been tested in a controlled prospective manner, however, due to frequent non-renal as well as renal adverse effects of NSAIDs. Highly dosed indomethacin not only placed the patients at risk for gastrointestinal bleeding, but many patients also did not tolerate these doses because of adverse effects on the central nervous system, e.g. non-orthostatic dizziness and somnolence. Another unwanted effect of NSAIDs comprises water and sodium retention, elevation of blood pressure and development of oedema, potentially annulling the renoprotective effects of proteinuria reduction. Finally, in patients with advanced renal function impairment, the use of NSAIDs is hampered by the decrease in GFR that accompanies an effective proteinuria reduction, as well as the propensity to hyperkalaemia due to specific tubular effects.
beneficial effect by other mechanisms, such as preventing thromboxane A2 (TXA2)-induced vasoconstriction or reducing inflammation. So far, high-dose aspirin combined with dipyridamole, in contrast to single aspirin treatment, has shown to be effective in reducing proteinuria accompanied by a stabilising effect on GFR in the long term in two different studies. The observed short-term antiproteinuric effect could not be explained by acute changes in GFR or ERPF. This may implicate a different mechanism of renoprotection as compared with ns-NSAIDs. The antiproteinuric effect of platelet-aggregating inhibitory agents seems predominantly mediated by blocking renal effects of TXA2, whereas the renoprotective effects of ns-NSAIDs are mediated by vasomodulatory effects of PGE2 inhibition.

COXIBS

As already mentioned, the antiproteinuric efficacy of NSAIDs relates to the extent of PGE2 inhibition, suggesting a pivotal role of PGE2 in the pathophysiology of kidney diseases. This is illustrated by ample evidence from murine models for renal disease. For example, COX-2-induced production of PGE2 induces mesangial expansion. Furthermore, upregulation of COX-2 not only increases susceptibility to podocyte injury, but also activation of the intrarenal RAAS leading to higher angiotensin II levels, i.e., processes that contribute to renal scarring and the development of proteinuria. Given the high frequency of ns-NSAID-related side effects, exploration of selective inhibition of COX-2 in renal disease was an obvious next step. Indeed, in the experimental setting coxibs had a renoprotective effect, as reviewed elsewhere, and improved responsiveness to ACE inhibitor therapy. Little is known of the effects of coxibs in human nephropathies. Only two studies tested the renoprotective potency of coxibs in proteinuric patients. We showed that rofecoxib reduced proteinuria by almost 30% in both diabetic and non-diabetic proteinuric patients. Patients were studied during a standard regimen of diuretic therapy and dietary salt restriction. In an additional protocol, short-term effects of rofecoxib (25 mg and 50 mg), indomethacin (150 mg retard formula), lisinopril (40 mg) and candesartan (32 mg) were compared in a cross-over fashion (figure 3). Rofecoxib 50 mg had a better antiproteinuric efficacy than 25 mg, but led to higher blood pressure and body weight, presumably due to sodium retention, and decrease of renal function. Furthermore, indomethacin, the ACE inhibitor lisinopril, and the ARB candesartan had a better antiproteinuric response than both doses of rofecoxib (figure 3A). In contrast, Sinsakul et al. could not confirm antiproteinuric efficacy of the coxib celecoxib in diabetic nephropathy. Their study was, however, not designed for specific exploration of celecoxib, as it was performed on a background therapy of RAAS blockade. Also, no measures to reduce volume excess were made, no dose-titration was performed, and no comparator drug was included in the protocol. In summary, the preliminary results indicate that coxibs have potential renoprotective characteristics by proteinuria reduction without blood pressure reduction.

ADVERSE EFFECTS OF NS-NSAIDS AND COXIBS

In general, the clinical application of coxibs and NSAIDs in renoprotective treatment schedules is hampered by safety concerns. As already mentioned, the use of NSAIDs is associated with water and sodium retention, consequent blood pressure elevation and oedema as well as hyperkalaemia. Also, renal function may deteriorate considerably, although the prevalence of renal toxicity in a non-renal population appears relatively low. Renal toxicity may particularly occur in clinical settings in which maintenance of renal blood flow and filtration pressure depends on prostaglandin synthesis to ensure afferent vasodilatation. This is the case during hypotension and/or decreased effective circulating volume, for instance due to heart failure, liver cirrhosis, or use of diuretics, and during age-related declines in GFR. Under those circumstances, NSAIDs can significantly decrease renal blood flow and filtration with resultant acute renal failure, usually functional and reversible upon restoration of circulating volume and withdrawal of the NSAID, but occasionally precipitating acute tubular necrosis. In addition, papillary necrosis and acute interstitial nephritis can occur in association with NSAIDs.

It is generally believed that renal effects of ns-NSAIDs and coxibs are similar, but the non-renal effects might differ. Coxibs are considered to have a more favourable gastrointestinal safety profile, due to their selectivity for COX-2. Furthermore, coxibs are related to adverse cardiovascular effects that led to immediate withdrawal of the coxib rofecoxib (Vioxx®) from the market after results from the APPROVe trial. The APPROVe trial studied rofecoxib in a non-renal population selected on a history of colorectal adenoma to prevent the development of recurrent neoplastic polyps, but was prematurely closed when rofecoxib at interim analysis was associated with an almost doubled risk of myocardial infarction and ischaemic cerebrovascular events. The elevated cardiovascular risk probably relates not solely to rofecoxib, but also to other coxibs, and to ns-NSAIDs. Yet, this relation seems rather heterogeneous, as some reports indicate that celecoxib may not share the coxib-related cardiovascular risk elevation. In particular, the elderly are at risk, also more frequently

Vogt, et al. NSAIDs in proteinuric kidney disease.
displaying congestive heart failure with a remarkable peak of heart failure exacerbations early after the start of the NSAID.45,46 A recent meta-analysis of 51 RCTs, comprising 130,541 patients, mainly suffering from osteoarthritis and rheumatoid arthritis, more frequently reported hypertension as a consequence of NSAID therapy.47 Particularly, the use of coxibs as compared with ns-NSAIDs was associated with markedly raised blood pressure. Based on these data from non-renal patients, the NSAID-related elevated cardiovascular risk seems for a great part attributable to blood pressure effects and heart failure. In renal patients, where some degree of volume retention is often already present, the use of ns-NSAIDs or coxibs might therefore be unattractive in conditions where volume excess is kept unattended, as this may not only elicit the above side effects but could also annihilate the antiproteinuric response.

**NSAIDs: A Role as Additive to Renoprotective Treatment Schedules?**

Given the rationale for adjunct antiproteinuric treatment in subjects with persistent proteinuria, the adverse effects of ns-NSAIDs and coxibs deserve proper attention when considering their use for renoprotective purposes. The risks should be weighed against the risks of persistent proteinuria in patients on an already optimised regimen based on RAAS blockade. It has consistently been shown that residual proteinuria is a strong predictor of the risk for progression towards end-stage renal disease, as well as for cardiovascular complications and death. As a rule of thumb, based on post-hoc RENAAL data, one could roughly expect a twofold elevation of the risk of a cardiovascular event for each 2 grams of proteinuria.6 In such a weighed risk model, acknowledgement of the elevated risk related to the wide accessibility of over-the-counter NSAIDs should be included too.24 Also, considering the effects of NSAIDs and coxibs on glomerular haemodynamics, which reduce autoregulatory capacity, in particular during concomitant RAAS blockade, and on renal sodium and potassium handling, such a regimen requires close monitoring of renal function, volume status and electrolytes, and should therefore only be used in dedicated nephrology settings.

If one decides to start with added NSAID therapy to RAAS blockade, patients should be instructed to seek medical attention in case of intercurrent dehydration (e.g., inadequate fluid intake, gastroenteritis, etc). Precautions to prevent the cardiovascular side effects related to volume retention apply to the use of ns-NSAIDs as well as coxibs, and consist of dietary salt restriction and/or diuretics, and inquiry about over-the-counter use of NSAIDs.24 Also, when the antiproteinuric response is absent or transient, one should be aware of volume retention as an underlying mechanism blunting therapeutic efficacy.44 We propose to use one of the still available coxibs as an additive measure when RAAS-inhibitor based treatment fails to reduce proteinuria sufficiently in the presence of normalised blood pressure, or leads to symptomatic hypotension. In this condition, the addition of a coxib might provide extra antiproteinuric efficacy by non-hypotensive action.

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**Figure 4. Proposal for a proteinuria remission regimen, including the use of coxibs.** Treatment goal should be proteinuria <1 g/day and blood pressure <125/<75 mmHg. The first step consists of the start with single RAAS blockade in combination with correction of volume overload. The second step comprises addition of another RAAS blocker on top of single ACE inhibitor or AT1 antagonist therapy, after treatment adherence has been checked. If the treatment goal is not reached or adverse events emerge (e.g. symptomatic hypotension, hyperkalaemia), the third step comprises dose tapering of RAAS blockade, addition of an antihypertensive agent from another class, and/or addition of an NSAID, preferably a coxib, under close monitoring.

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Vogt, et al. NSAIDs in proteinuric kidney disease.
Although ns-NSAIDs could theoretically have advantages above coxibs related to their non-selective inhibition of both COX-1 and COX-2, coxibs are better tolerated by patients than highly dosed indomethacin. Also, coxibs have a lower risk of gastrointestinal bleeding. Moreover, in order to prevent such bleeding complications, application of a highly dosed combination of platelet aggregating inhibitory agents would not be attractive in remission regimens. Figure 4 shows an individually tailored remission regimen, including correction of volume excess by low sodium intake and diuretic use, dose titration with single RAAS blockers, dual RAAS blockade, lipid control, and newer proposed renoprotective interventions, i.e. mineralocorticoid blockade and renin inhibition.

CONCLUSION

Regarding the markedly elevated cardiovascular and renal risk in patients with inadequately treated proteinuria, the antiproteinuric effect of NSAIDs may outweigh the adverse effects of NSAIDs. Clearly, the use of NSAIDs for the purpose of renoprotection can act as a two-edged sword and, therefore, more prospective evidence is needed to verify the assumption that lowest proteinuria obtained by the addition of NSAIDs leads to beneficial long-term renal and cardiovascular outcome. For patients with a high risk of proteinuria-driven progression to ESRD, despite adequate RAAS blockade, the odds of using NSAI or coxibs as an additive measure to reduce proteinuria in a non-hypotensive way may be favourable. The combination of RAAS blockade with NSAIDs or coxibs for proteinuria is a powerful, but risky combination, and for its possible benefits to be realised, a dedicated setting and close monitoring are required.

REFERENCES


Vogt, et al. NSAIDs in proteinuric kidney disease.
Acute Q fever related in-hospital mortality in the Netherlands


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ABSTRACT

Introduction: A large outbreak of acute Q fever has been reported in the Netherlands with over 3500 cases from 2007 to 2009, during which 749 patients were hospitalised. In foreign cohorts, reported mortality rates in patients hospitalised with acute Q fever, ranged from 0.9 to 2.4%. We analysed mortality among hospitalised patients with acute Q fever in the Netherlands.

Methods: Physicians from hospitals in the afflicted region were asked to provide details about patients who died with a diagnosis of acute Q fever between 2007 and 2009.

Results: Nine patients (seven males, median age 72 years) from six hospitals were reported, who died within approximately one month following hospitalisation for acute Q fever. Six definite acute Q fever cases and three probable cases were identified. Six patients presented with infiltrates on the chest X-ray and a median CURB-65 score of 3. Median time of hospitalisation was 13 days (range 1-33). All patients had serious, often coinciding, underlying conditions including chronic cardiovascular disease, chronic lung disease, diabetes mellitus and malignancy.

Conclusion: The mortality rate of patients hospitalised because of acute Q fever was estimated at approximately 1%. Patients who died with acute Q fever were often male, of older age, and had chronic coinciding underlying conditions, which gives an a priori higher risk of death.

KEYWORDS

Coxiella burnetii, hospitalisation, mortality, Q fever

INTRODUCTION

Q fever is a zoonotic infection, caused by *Coxiella burnetii*, an intracellular gram-negative coccobacillus. There is a large animal reservoir, with goats, sheep and cattle being the most common source of human infections, although infections from birds, pets and arthropods have also been described. When infected, mammals shed *C. burnetii* in urine, faeces, milk and especially birth products. In placental tissues of infected animals, up to 10^9 microorganisms per gram of tissue can be found. Humans get infected from direct contact with infected animals and/or inhalation of contaminated aerosols. Most people infected with *C. burnetii* did not have close contact with infected animals, but were infected because of windborne spread of bacteria, which can travel over several kilometres. Rarely, people get infected from drinking contaminated milk and sporadic human-to-human transmission has been described following contact with an infected parturient woman, blood transfusion or sexual intercourse.

Q fever has both acute and chronic manifestations and the presentation of the disease is extremely variable. After infection, most patients (50 to 60%) remain asymptomatic. Typically, symptomatic patients report a flu-like illness with fever, myalgia, fatigue, headache and artralgia, often accompanied by respiratory signs of pneumonia. Mild elevations of transaminases can be present, and severe acute hepatitis may occur. More rarely, pericarditis, myocarditis, meningitis, peripheral neuropathy and haemolytic anaemia accompany an acute Q fever infection. Symptoms can last from ten to 90 days, and usually resolve spontaneously. Antibiotic treatment with doxycycline or fluoroquinolones...
is only warranted in symptomatic patients to shorten the duration of the fever and to hasten recovery from the pneumonia. A significant number of acute Q fever patients subsequently develop a chronic fatigue syndrome, which can last five to ten years after the acute illness. Following an acute infection with C. burnetii, 1 to 5% of patients progress to chronic infection, which can even develop years after the primary infection. Endocarditis, vascular aneurysm and prosthesis infection are the most common manifestations. Most frequently affected are patients with pre-existent valvular disease and vascular defects (especially aortic aneurysms and aortic stents/prosthesis), immunocompromised patients and pregnant women.

Diagnosis of Q fever mandates notification to the municipal health authorities in the Netherlands. In the last three consecutive years, there has been a large expanding outbreak of Q fever in the south of the Netherlands: in 2007, a small outbreak of 168 cases was identified, while in 2008 and 2009, the epidemic progressed to 1000 and 2357 cases respectively.

According to the literature, rates of hospital admission in symptomatic patients with acute Q fever range from 2% to as high as 63%. Reported overall mortality rates of acute Q fever range from 0.5 to 2% in French and Australian populations. Mortality data for hospitalised patients with acute Q fever range from 0.9 to 2.4% and are available from older reports from the United Kingdom (1979) and France (1992), respectively.

In the Netherlands, 749 patients are known to the national health services to have been hospitalised with acute Q fever from 2007 to 2009. This number is presumably not completely accurate, as it is extracted from questionnaires sent to the general practitioners of notified acute Q fever patients. Extrapolation of the previously published mortality rates for hospitalised patients with acute Q fever allows for an estimation of seven to 18 deaths in this three-year period in the Netherlands. However, although Q fever itself has been a notifiable disease in the Netherlands since 1978, there is no requirement to notify deaths attributable to this disease. Therefore, there are no accurate data on mortality rates during the Q fever epidemic in the last three years in the Netherlands. In the present report, we assess the mortality rate among hospitalised patients with an acute Q fever infection and, in addition, evaluate epidemiological characteristics of these patients. Death due to chronic Q fever has not yet been evaluated as it can be expected that this condition still has to develop in a significant number of patients at risk.

METHODOLOGY

Q fever in the Netherlands is mostly restricted to the middle and southern areas of the country. By October 2009, clinicians and microbiologists from 12 hospitals in the afflicted regions were asked to provide details about patients who were admitted at their hospital and died with a diagnosis of acute Q fever. If an acute Q fever-related death was reported, we requested information about patient characteristics, comorbidity, performed diagnostic procedures (chest X-ray, polymerase chain reaction (PCR), serology), severity of pneumonia and antimicrobial treatment.

Until 2008, laboratory diagnosis of acute Q fever in the Netherlands was mainly established by serological testing for antibodies to phase I and phase II antigens of C. burnetii. The most commonly used tests are the indirect immunofluorescence assay (IFA; Focus Diagnostics, Inc., Cypress, CA, USA), complement fixation test (CFT; Siemens Healthcare Diagnostics GmbH, Eschborn, Germany) and enzyme-linked immunosorbent assay (ELISA; Institut Virion)Serion GmbH, Würzburg, Germany). Appearance of phase II IgM and IgG antibodies indicates an acute Q fever infection. Seroconversion usually takes place seven to 15 days after onset of clinical symptoms.

Since 2009, PCR on serum has become an important tool in the diagnosis of Q fever. PCR for C. burnetii allows the diagnosis of acute Q fever early after onset of disease, before seroconversion has taken place. Adhering to recently published Dutch guidelines, the diagnosis of acute Q fever was considered definitive on the basis of either a positive serum PCR or a seroconversion or fourfold increase in antibody titres to C. burnetii as detected by either IFA or CFT in two consecutive serum samples. The diagnosis was considered possible when there were clinical signs compatible with an acute Q fever infection in concordance with the presence of antibodies to C. burnetii as detected by either IFA or CFT in a single serum sample.

When pneumonia was suspected and confirmed on chest X-ray, the CURB-65 score was used as an index for the severity of pneumonia. The CURB-65 score is a clinical method of predicting the mortality of community-acquired pneumonia (CAP). It consists of five criteria, scoring 1 point each: confusion of new onset, urea >7 mmol/l, respiratory rate ≥30 breaths, blood pressure systolic <90 mmHg or diastolic ≤60 mmHg and age ≥65 years. A CURB-65 score of 0 gives a less than 1% 30-day mortality risk, score of 1 a 3% risk, score of 2 a 13% risk, score of 3 a 17% risk, score of 4 a 42% risk and score of 5 a 57% risk.

RESULTS

Survey results
Nine patients who had died following hospitalisation with acute Q fever were identified: seven males and two females (78 vs 22%), admitted in six different hospitals. All patients were at least 55 years or older. Median age at
Time of death was 72 years (range 55 to 86). All patients had serious, often coinciding, underlying conditions including chronic cardiovascular disease (five patients; 56%), chronic lung disease (seven patients; 78%), diabetes mellitus (four patients; 44%) or malignancy (two patients; 22%) (Table 1). Median time of hospitalisation before death was 13 days (range 1 to 33). There were no reported deaths due to acute Q fever in 2007. Two patients died in 2008 and seven patients in 2009 (Figure 1).

Clinical and microbiological diagnosis of acute Q fever

An overview of the clinical and microbiological features is presented in Table 1. Six patients presented with evident infiltrates on chest X-ray and a median CURB-65 score on admission of 3 (range 1 to 3). The other three patients had no evident infiltrative changes on the chest X-ray, and as a result no CURB-65 score was calculated. One of them suffered from acute myeloid leukaemia and chemotherapy-induced neutropenia, which could explain the absence of infiltrative changes. Another patient suffered from metastatic lung carcinoma hindering detection of any infiltrative changes. Median C-reactive protein (CRP) level and white blood cell count (WBC) at hospital admission were 100 mg/l (range 43 to 267) and 10.2 x 10^9/l (range 4.0 to 14.0, after exclusion of the patient with chemotherapy-induced neutropenia), respectively.

A definite laboratory diagnosis of acute Q fever was made by positive serum PCR for C. burnetii in four

<table>
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<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Days in hospital</th>
<th>CURB-65 score</th>
<th>Comorbidity</th>
<th>Antimicrobial treatment before diagnosis of Q fever</th>
<th>Antimicrobial treatment after diagnosis of acute Q fever</th>
<th>Other pathogens, besides C. burnetii</th>
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<td>Doxycycline, vancomycin, co-trimoxazole, voriconazole and aciclovir</td>
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M = male; F = female; PCR = polymerase chain reaction; IFA = indirect immunofluorescence assay; CFT = complement fixation test; COPD = chronic obstructive pulmonary disease; CABG = coronary artery bypass graft; CVA = cerebral vascular accident; ESBL = extended-spectrum beta-lactamase.
seronegative patients and two seropositive patients. In one patient, who died one day after hospital admission, the definite diagnosis of acute Q fever was established four months post-mortem through PCR on a stored seronegative serum sample. In three patients with clinical signs compatible with an acute Q fever infection, a laboratory diagnosis of possible acute Q fever was made on the basis of positive serology in a single serum sample. As these patients died nine to 13 days after hospital admission, a second serum sample to confirm the diagnosis could not be obtained. Both patients with a definite and a possible diagnosis were included in the overall analysis.

Antimicrobial treatment
Table 1 gives an overview of the prescribed antimicrobials and co-pathogens for the nine patients who died with an acute Q fever infection. Five patients were initially treated with antibiotics with proven activity against C. burnetii. The sixth patient switched after two days and the seventh patient after four days of admission to an antibiotic with proven activity against C. burnetii, before the actual diagnosis was made. After diagnosis of acute Q fever, antibiotic treatment was switched from ciprofloxacin to doxycycline in one patient and from doxycycline to moxifloxacin in a second patient, while in a third patient doxycycline was added to co-trimoxazole. In the two patients who were never treated with an adequate antibiotic regime for C. burnetii, the diagnosis of acute Q fever was made post-mortem.

In cultures of four patients, other pathogens were detected, which influenced the choice of antimicrobial treatment.

DISCUSSION
We identified nine patients who died, within approximately one month following hospital admission, with definite or possible acute Q fever in the period of 2007 to October 2009. With 749 known hospital admissions due to acute Q fever in the Netherlands from 2007 to 2009, the in-hospital mortality rate is approximately 1%, which is relatively low and illustrative of the relatively mild nature of the acute form of this disease. In comparison, the reported overall in-hospital mortality rate of CAP in the Netherlands is 8%. Seven out of the nine patients were males. This is in line with the fact that male sex is a risk factor for symptomatic acute Q fever and the reported incidence of acute Q fever in males and females. In surveys from Australia and France, males are fivefold and 2.5-fold, respectively, more likely to develop symptomatic acute Q fever. Doxycycline and fluoroquinolones are the antibiotics of choice for acute Q fever. Treatment lessens the duration of fever and hastens recovery of pneumonia, but the effect on mortality has not been investigated. Initiation of treatment three days after symptom onset is reported to be less effective; however, good clinical responses have been observed with treatment up to a week from start of symptoms. Five out of nine patients were initially treated with adequate antibiotic regimes for acute Q fever. Two patients started adequate therapy at admission-day two and four, respectively.

All nine patients had serious, often coinciding, underlying conditions including chronic cardiovascular disease, chronic lung disease, diabetes mellitus and malignancy.
In four patients, co-pathogens, besides *C. burnetii*, were detected. It is feasible that these pathogens contributed to some extent to the patients' death and influenced the choice of antimicrobial therapy. In addition, six patients were older than 65 years, which is one of the risk factors that stratifies patients with CAP to a higher risk class in the CURB-65 score. With a score of 3, the median CURB-65 score in the six patients who presented with pneumonia was relatively high, representing a 30-day mortality risk of 17%.

In comparison, median age at time of hospitalisation of 28 patients with non-lethal acute Q fever who had been included in 2008 to 2009 in a prospective observational study on the aetiology of CAP at the Jeroen Bosch Hospital was 55 (range 23 to 96; 25% of patients older than 65 years). This cohort consisted of 19 males and 9 females (68 vs 32%). Median CURB-65 score on admission was 0 (range 0 to 4) and median time of hospitalisation was six days (range 2 to 14). Overall, patients in this cohort had far less relevant underlying conditions (three patients (11%) with chronic cardiovascular disease, five patients (18%) with diabetes mellitus, one patient with chronic hepatitis C and one patient with chronic use of methotrexate for chronic arthritis, while no patients with chronic lung disease or malignancy were identified; unpublished data). Likewise, an earlier Dutch report by de Wit et al. of 25 non-lethal, hospitalised, acute Q fever cases in the Netherlands, described a CURB-65 score of 0±1 (mean ± SD). These observations indicate that hospitalised patients who eventually died of an acute Q fever infection were at time of presentation already more severely ill than patients who survived.

The aim of this report was to make an estimation of in-hospital acute Q fever related-deaths in the Netherlands and to describe the patient characteristics of the fatalities. It is feasible that death as a result of acute Q fever is underreported. There are as yet no adequate databases of hospitalised acute Q fever patients in the Netherlands and the exact number of patients hospitalised due to acute Q fever is not known. Also, there is no requirement to notify deaths attributable to this disease. Furthermore, it is more than likely that due to its design, our survey was not all-comprehensive. In most hospitals, PCR for *C. burnetii* was not introduced until early 2009. It is, therefore, possible that in 2007 and 2008, seronegative patients may have died from pneumonia or febrile disease caused by *C. burnetii*, in whom the diagnosis could not be established with PCR. These patients are missed in this survey. This is illustrated by the fact that in one seronegative case, diagnosis was made through PCR four months post-mortem. However, even if death to acute Q fever is underreported, this also holds true for non-fatal cases of the disease. For example, Schneebberger et al. have previously shown that retrospective PCR analysis on stored serum samples from patients in whom the diagnosis of acute Q fever had not been made using serological techniques allowed this diagnosis to be made in 5/50 (10%) cases. Moreover, especially in 2007 and 2008, many clinicians were still unaware of the existence of a Q fever epidemic. Since there is an evident overlap in symptoms with other febrile diseases, the possibility of acute Q fever could easily be overlooked and, subsequently, no diagnostic tests to detect *C. burnetii* were ordered. Thus, death to acute Q fever as well as acute Q fever itself might be underreported, warranting some caution towards the in-hospital mortality rate reported in this survey.

**CONCLUSION**

The in-hospital mortality rate of acute Q fever in the Netherlands can be estimated at around 1%, which is relatively low compared with the overall in-hospital mortality rate of CAP and illustrates the relatively mild nature of the acute form of this disease. Patients who died with acute Q fever were often male, of older age, and had chronic coinciding underlying conditions, which gives an a priori higher risk of death. The rate of death cannot be accurately defined, because there is no obligation to register Q fever-related admissions and fatalities in the Netherlands. Better registration is necessary to provide a detailed estimation of mortality because of acute Q fever.

This survey was presented at the Dutch Society for Microbiology/Dutch Society for Infectious Diseases autumn meeting in November 2009.

**REFERENCES**


A rare cause of obstructive jaundice and weight loss in Von Recklinghausen’s disease

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ABSTRACT

We present the case of a patient with the rare triad of Von Recklinghausen’s disease associated with a somatostatinoma and a gastrointestinal stromal tumour (GIST). The patient had recurrent jaundice, the typical somatostatinoma syndrome, positive MR imaging but negative 68Ga-DOTATOC PET scanning in a histopathology-proven somatostatinoma tumour.

KEYWORDS

68Ga-DOTATOC PET scan, gastrointestinal stromal tumour, MR imaging, somatostatinoma, Von Recklinghausen’s disease

CASE REPORT

A 48-year-old male with a history of Von Recklinghausen’s disease presented with recurrent, spontaneously resolving jaundice, diarrhoea and weight loss of 6 kg. Laboratory results showed serum bilirubin levels varying between 50 µmol/l and 165 µmol/l (normal <17 µmol/l) with corresponding fluctuations in cholestatic liver enzymes. Ultrasonography and CT scanning revealed a markedly dilated gallbladder (calculated fasting volume 240 ml; normal <50 ml) with sludge and multiple concrements, dilated intra- and extra-hepatic bile ducts, and a hypodense mass at the pancreatic head, near the distal common bile duct and duodenal wall. Additional lab investigations demonstrated marked steatorrhoea with faecal fat excretion of up to 90 g/day. Blood glucose levels were mildly elevated (9.2 mmol/l).

Upper gastrointestinal endoscopy revealed a polypoid mass around the papilla of Vater. Immunohistochemical staining of biopsy specimens revealed a somatostatinoma (figure 1). A gastric emptying study with scintigraphy performed after the diagnosis of somatostatinoma showed delayed gastric emptying for a solid meal with t½ of 103 min (normal values: 53 to 79 min). The somatostatinoma was functionally active.

Figure 1. During duodenoscopy, biopsies were taken from the polypous tissue near the papilla of Vater. After standard fixation and processing of the polypous tissue, immunohistochemistry with antisomatostatin antibody (somatostatin stain, red brown) was performed. Intense cytoplasmic labelling of somatostatin is evident and multiple psammoma bodies are identified (original magnification, x20).
with an inhibitory syndrome (delayed gastric emptying, gallbladder stasis, diarrhoea and steatorrhoea, weight loss, diabetes mellitus). Plasma somatostatin concentrations were measured and appeared to be elevated, under fasting and fed conditions with values of 400 pmol/l and 600 pmol/l, respectively (normal value <150 pmol/l). The postprandial increase in serum levels of gastrointestinal peptides such as gastrin and pancreatic polypeptide (PP) was suppressed (normal postprandial increase of gastrin and PP: 40 to 100 pmol/l) and may have resulted from the inhibitory effect of somatostatin (figure 2).

Additional MR imaging showed a large mass in the ampullary region extending to the pancreatic head (figure 3A and B). A 68Ga-DOTATOC PET scan was performed for staging and detection of possible metastases. 68Ga-DOTATOC (tracer) is a somatostatin receptor agonist which reflects the expression of somatostatin receptors on the tumour surface. The 68Ga-DOTATOC PET scan showed a lack of abnormal tumoural 68Ga-DOTATOC uptake (figure 3C). Co-registered MR-PET imaging showed 68Ga-DOTATOC hyperactivity in the kidneys because of physiological excretion, but no specific hyperactivity at the site of the tumour (figure 3D).

During laparotomy, a lesion in the ampullary region extending to the pancreatic head was palpated. In addition, a 0.6 x 0.6 cm tumour was observed on the outer surface of the jejunum. A pylorus preserving pancreaticoduodenectomy and resection of the jejunal tumour was performed. Histological examination revealed a somatostatinoma in the pancreatic specimen with a diameter of 3.5 cm and one positive lymph node (pT 3N1Mx stadium). The presence of a high amount of somatostatin was confirmed by immunohistochemistry. The jejunal lesion with CD117/c-kit expression revealed a gastrointestinal stromal tumour (GIST) (figure 4). Postoperative recovery was uneventful. After a follow-up period of two years (clinical follow-up every six months and yearly CT imaging), we have not observed any sign of recurrence.

**DISCUSSION**

Somatostatinomas are malignant, somatostatin-producing neuroendocrine tumours (NETs). They are rare with an estimated annual incidence of one per 40 million and a median age at onset of 54 years. In over 90% of patients these tumours are symptomatic.1 The classical syndrome in a patient with a somatostatinoma is characterised by diabetes mellitus, cholelithiasis, diarrhoea with steatorrhoea and hypo- or achlorhydria resulting from the inhibitory actions of somatostatin on gastrointestinal motility and secretion. Somatostatin inhibits the secretion and the action of various regulatory peptides and hormones, such as insulin, cholecystokinin (CCK), glucagon, gastrin, and PP. Thereby, motor and secretory functions as gallbladder contraction, pancreatic enzyme secretion, and gastric acid secretion are impaired.4

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**Figure 2.** Results of a nutrition challenge test: Plasma somatostatin, gastrin and pancreatic polypeptide (PP) levels, fasting and after ingestion of a fat-rich 600 kCal meal. The meal was ingested at time 0 min. Fasting and postprandial plasma somatostatin is increased (normal value <150 pmol/l). The postprandial response of gastrin and PP is impaired (normal postprandial increase of gastrin and PP: 40 to 100 pmol/l)

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**Figure 3.** T1-weighted MR image shows a large mass (with low signal intensity, arrow) in the ampullary region extending to the pancreatic head (A). Post-gadolinium contrast enhanced T1-weighted MR imaging shows an intense arterial enhancement of the tumour (arrow), which reflects the abundant blood supply characteristic of neuroendocrine tumours (B). Transverse 68Ga-DOTATOC PET (C) and co-registered MR-PET image (D) show only specific uptake in the kidneys but not in the somatostatinoma tumour area

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**Figure 4.** Immunohistochemical stain for CD117/c-kit (brown) and Ki67 (red) in the jejunal tumour (left) and the somatostatinoma (right)
The incidence of a GIST is 4 to 22 per 1,000,000. Somatostatinomas as well as GISTs are increasingly being recognised in association with Von Recklinghausen’s disease. Of the somatostatinomas that have been reported to originate in the peri-ampullary region 40% were associated with Von Recklinghausen’s disease. GISTS occur in up to 3.9 to 25% of patients with Von Recklinghausen’s disease.14 The prevalence of Von Recklinghausen’s disease in patients with GIST is up to 6%.3 A review of the literature revealed seven previously reported cases of this rare clinical triad presented here.3,4,6-10 Most NETs demonstrate low signal intensity on T1-weighted images and high signal intensity on T2-weighted images. Moderate or intense early enhancement of portions of the primary NETs reflects the abundant blood supply of these tumours.11-13 The complete spectrum of MR imaging (figure 3) confirms the above-mentioned imaging features. Positive and negative predicted values for MR imaging in the detection of 22 NETs reported by Semelka et al. were 96 and 100%, respectively.11 For this reason, we suggest a more prominent future role for MR imaging in the diagnosis and evaluation of suspected NETs.

68Ga-DOTATOC PET is increasingly used as it is superior for the detection of NETs compared with conventional somatostatin receptor scintigraphy (SRS) with SPECT and diagnostic CT.14,15 Evaluation of the diagnostic value of 68Ga-DOTATOC PET compared with SRS with SPECT and CT in 84 patients with known or suspected NETs by Gabriel et al. resulted in 97% sensitivity for PET, 52% for SPECT and 61% for CT; 92% specificity for PET, 92% for SPECT and 71% for CT and 96% accuracy for PET, 58% for SPECT and 63% for CT. These differences in diagnostic efficacy were statistically significant in favour of 68Ga-DOTATOC PET (p<0.001). The combined use of PET and CT showed the highest accuracy.15 DOTATOC PET scanning is important in the preoperative work-up as it provides information on possible metastases. Although several authors reported positive SRS and 68Ga-DOTATOC PET scans in cases of somatostatinomas, the tumour in our patient lacked uptake on the DOTATOC scan.19-27 We postulate that the reason for the absence of radionlabelled somatostatin uptake in our case may be due to locally high somatostatin concentrations that resulted in either down-regulation of the somatostatin receptors on the tumour surface reducing the binding potential for the small fraction of radionlabelled DOTATOC, or in competitive binding with the labelled somatostatin.

CONCLUSION

Although somatostatinomas and GISTS are rare tumours, they should be considered in patients with Von Recklinghausen’s disease with unexplained gastrointestinal symptoms. Our patient had a functional somatostatinoma with clinically overt inhibitory somatostatinoma syndrome. Although 68Ga-DOTATOC PET is superior for the detection of NETs compared with SPECT and CT, the tumour in our patient lacked uptake of 68Ga-DOTATOC. Diagnostic accuracy of MR imaging in detecting NETs is promising and combined with 68Ga-DOTATOC PET scanning may provide the most accurate diagnostic tool for preoperative staging of gastrointestinal neuroendocrine tumours, including somatostatinomas.

ACKNOWLEDGEMENTS

We thank our colleagues B. Brans, for his critical revision of the manuscript and his useful comments and Ms. N. Helgers, for developing the co-registered MR-PET image (both working at the Department of Nuclear Medicine at Maastricht University Medical Centre, the Netherlands).

REFERENCES

Figure 4. After standard fixation and processing of the jejunal tissue, immunohistochemistry with anti-c-kit protein (CD117) was performed. CD117 stain is the product of the c-kit proto-oncogene that encodes a tyrosine-kinase receptor, which is responsible for cellular proliferation in GISTs. Positive CD117 stain (red-brown) revealed a gastrointestinal stromal tumour (original magnification, x20).
Histoplasma capsulatum reactivation with haemophagocytic syndrome in a patient with chronic lymphocytic leukaemia

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ABSTRACT

We describe a case of haemophagocytic syndrome caused by Histoplasma capsulatum reactivation in a patient with chronic lymphocytic leukaemia treated with fludarabine and alemtuzumab. He presented with fever, pancytopenia, increased serum ferritin, lactate dehydrogenase and soluble interleukin-2 receptor. A bone marrow aspirate showed haemophagocytosis and possibly a yeast infection. Treatment with cyclosporine, dexamethasone, etoposide and caspofungin was started. After initial improvement his condition deteriorated. A second bone marrow examination confirmed a Histoplasma infection. After treatment with amphotericin B, the fever resolved and blood counts normalised. Haemophagocytic syndrome is a critical condition with high mortality that requires immunosuppressive therapy. The underlying cause should be investigated and treated. In this case a Histoplasma reactivation is described in a severely immunocompromised host years after the patient had left the endemic area.

KEYWORDS

Histoplasma, haemophagocytic syndrome, chronic lymphatic leukaemia

INTRODUCTION

Haemophagocytic syndrome or haemophagocytic lymphohistiocytosis (HLH) is a potentially lethal condition caused by inappropriate activation and proliferation of lymphocytes and macrophages with an uncontrolled immune response leading to cellular damage in multiple organ systems causing pancytopenia, hyperferritaemia and hepatosplenomegaly. It has been classified into a primary or genetic form and a secondary or reactive form, which is associated with a variety of infections, autoimmune diseases and malignancies (table 1). We describe a case of HLH induced by a systemic Histoplasma reactivation in a severely immunocompromised patient with chronic lymphatic leukaemia (CLL).

Table 1. Classification of haemophagocytic syndromes

<table>
<thead>
<tr>
<th>Primary haemophagocytic syndrome</th>
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<tr>
<td>Familial haemophagocytic syndrome type 1-4</td>
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<tr>
<td>Hereditary immune deficiencies</td>
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<td>Griscelli type 2 syndrome</td>
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<td>Chediak-higashi syndrome</td>
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<tr>
<td>X-bound lymphoproliferative syndrome</td>
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<tr>
<td>Hermansky-Pudlak type 2 syndrome</td>
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<td>Autoimmune proliferative syndrome</td>
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<tr>
<th>Secondary haemophagocytic syndromes</th>
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<tbody>
<tr>
<td>Infections</td>
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<tr>
<td>• Viral (Herpes, HIV, Hepatitis, etc)</td>
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<tr>
<td>• Bacterial (Mycobacteria, Mycoplasma, Chlamydia)</td>
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<tr>
<td>• Fungal (Aspergillus, Candida, Histoplasma)</td>
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<tr>
<td>• Parasitic (Falcipurum falciforme)</td>
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<tr>
<td>Autoimmune diseases</td>
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<tr>
<td>• Systemic lupus erythematoses</td>
</tr>
<tr>
<td>• Reumatoid arthritis</td>
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<tr>
<td>• Still’s disease</td>
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<tr>
<td>• Polyarteritis nodosa</td>
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<tr>
<td>• Sjogren’s disease</td>
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<tr>
<td>• Mixed connective tissue disease</td>
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<tr>
<td>• Scleroderma</td>
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<tr>
<td>Malignancies</td>
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<tr>
<td>• Natural-killer, B- and T-cell lymphoma</td>
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<tr>
<td>• Leukaemia</td>
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<tr>
<td>Chemotherapy</td>
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A 50-year-old man presented with shivering and relapsing fever for two weeks. He had no other physical complaints. Five years before he was diagnosed with CLL with slowly progressing generalised lymphadenopathy. Seven months before admission he developed anaemia and thrombocytopenia with extensive CLL bone marrow infiltration. Treatment with immunochemotherapy was initiated according to the HOVON 68 protocol with alemtuzumab, fludarabine and cyclophosphamide. The fever started two months after the fifth cycle. Born in Suriname, he had been living in the Netherlands since childhood and had not visited his country of birth again. It had been two years since he had been travelling to urban areas in Oman and Qatar, Malaysia and Thailand. Physical examination was unremarkable except for a temperature of 39.8 °C. Laboratory tests revealed pancytopenia with a haemoglobin of 7.6 mmol/l (normal range (N) 8.7 to 10.9 mmol/l), thrombocytes 112 10^9/l (N 150 to 400 10^9/l), leukocytes 1.4 10^9/l (N 4.5 to 11 10^9/l), alanine aminotransferase 58 U/l (N1 to 41 U/l), lactate hydrogenase (LDH) 510 U/l (N <270 U/l) and a C-reactive protein (CRP) of 82 mg/l (N <10 mg/l). Extensive laboratory tests for viral, bacterial or fungal infections were performed with negative results (blood cultures, serology for Varicella Zoster, HIV, Hepatitis A,B,C, Adenovirus, Parvo B19 virus, Chlamydia, Mycoplasma, Coxiella and whole blood polymerase chain reaction (PCR) for Epstein Barr virus and cytomegalovirus, and bone marrow PCR for tuberculosis). A computed tomography (CT) scan of the neck, chest and abdomen showed stable enlarged parajugular and para-aortal lymph nodes, no hepatosplenomegaly and no focus of infection. A bone marrow examination showed granulomatous inflammation with suspicion of a yeast infection and signs of haemophagocytosis. The soluble interleukin-2 (IL2) receptor serum concentration was 13,410 pg/ml (N <2500 pg/ml), ferritin was 510 µg/l (N 22 to 270 µg/l) and triglycerides were 2.33 mmol/l (N <2 mmol/l). A diagnosis of HLH was made (table 2) possibly secondary to a yeast infection or chemotherapy. Treatment was initiated according to the Histiocyte Society 2004 protocol with daily oral dexamethasone and cyclophosphamide, and etoposide twice a week intravenously. Continuous intravenous caspofungin was added to treat the yeast infection. Initially the fever quickly disappeared with clinical improvement but ten days after starting treatment, the fever relapsed. Serum ferritin levels and LDH increased and pancytopenia worsened after an initial improvement (figure 1). Clinical examination, blood and urine cultures, and repeated chest CT scan revealed no focus of infection. A second bone marrow examination showed extensive haemophagocytosis and a yeast morphologically resembling Histoplasma capsulatum (figure 2). Later this was confirmed by PCR and culture. Caspofungin was switched to amphotericin B intravenously and the fever disappeared. Also ferritin and LDH levels decreased followed by the soluble IL2 (figure 1). After five weeks of treatment cyclophosphorine was stopped and the dexamethasone was tapered. After two weeks of intravenous amphotericin B therapy the patient was placed on oral itraconazole and was discharged. Six months after discharge he was in excellent clinical condition with normal blood counts, LDH and ferritin.

Table 2. Histiocyte Society 2004 diagnostic criteria for HLH (5 or more criteria should be fulfilled)

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Value</th>
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<tr>
<td>Fever (&gt;38.5 °C for at least 7 days)</td>
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<tr>
<td>Splenomegaly</td>
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<td>Cytopena (at least 2 of 3 cell lines)</td>
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<tr>
<td>Hypertriglyceridaemia and/or hypofibrinemia</td>
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<tr>
<td>Haemophagocytosis in bone marrow, spleen or lymph nodes, without signs of malignancy</td>
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<tr>
<td>Ferritin 2500 µg/l</td>
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<tr>
<td>Soluble IL2 receptor (Soluble CD25) ≥2500 pg/ml</td>
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<tr>
<td>Low or absent NK-cell activity</td>
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DISCUSSION

Haemophagocytic lymphohistiocytosis presents diagnostic and therapeutic challenges. As symptoms may be non-specific together with the rarity of the disease, HLH is often diagnosed in a late phase. In our patient who presented primarily with fever and pancytopenia the haemophagocytic syndrome was recognised relatively early in the first bone marrow examination. Furthermore, a yeast infection was suggested in this bone marrow specimen. Extensive microbiological investigation for other possible underlying infections delivered negative results. Immunochemotherapy and antifungal therapy with Candida and Aspergillus species coverage was started. After a short period of clinical improvement, the HLH relapsed. A second bone marrow biopsy showed HLH and Histoplasma capsulatum infection. This yeast proved to be resistant to caspofungin and after switching to amphotericin B the HLH was controlled.

When left untreated, primary HLH patients rarely survive; however, since implementation of immunochemotherapeutic interventions survival has increased to more than 50%. Infection-associated HLH mortality has been estimated at 52 to 73%. Treatment is aimed at suppression of the uncontrolled, inappropriate inflammatory response and elimination of the underlying cause (table 1). Our patient was treated according to the Histiocyte Society 2004 protocol with a combination of dexamethasone, cyclosporine for lymphocyte specific toxicity and etoposide for its antimacrophage action. Although treatment of the underlying infection alone has been associated with recovery in 60 to 70% of patients, immunosuppressive therapy is recommended rather than antimicrobial monotherapy in infection-related HLH. Our patient did not complete the Histiocyte Society protocol because of worsening pancytopenia after two gifts of etoposide. This was stopped and dexamethasone and cyclosporine were continued for a total of five weeks (figure 1). As no randomised studies concerning secondary HLH treatment have been performed, there is no golden standard for therapy. As the clinical course of our patient showed, treatment of the underlying cause of HLH is of utmost importance. Histoplasma capsulatum associated HLH has been described before in association with HIV infection renal and heart transplant recipients, patients on prolonged corticosteroid therapy for sarcoidosis or hepatitis C and a few non-immunocompromised patients. However, histoplasmosis is rarely diagnosed in northern Europe. Histoplasma is endemic in the Mississippi River valley, Central and South America and has been found frequently in southern Europe, South-East Asia and Africa. Histoplasma is a dimorphic fungus that behaves like a yeast at 37 °C and can survive in soil like a mould. Infection occurs through inhalation of microconidia formed in the mould phase. In the lung the organism is phagocytised by macrophages, converts to a yeast form inside the macrophage and is transported through the reticulo-endothelial system. After weeks cellular immunity mediated by T-helper cells is acquired and macrophages are activated to kill the microorganism. Less than 1% of primarily infected subjects will develop clinical illness: a self-limiting pneumonia with fever, malaise, headache and a dry cough. Older patients are at risk for chronic cavitary pulmonary histoplasmosis. Disseminated histoplasmosis including sepsis and HLH occurs almost exclusively in immunocompromised patients. As with other intracellular microorganisms Histoplasma can remain latent in macrophages. When cell-mediated immunity is suppressed, reactivation can cause disease even decades after leaving the endemic area.

CONCLUSION

Histoplasma capsulatum associated HLH is a rare but potentially dangerous cause of fever in immunocompromised patients. Bone marrow examination, ferritin and soluble IL2 receptor are the diagnostic tests of choice. Extensive microbiological testing for underlying infectious causes should be performed. Treatment consists of immunomodulation and elimination of the underlying infection. In this case determination of the infective agent was critical. In addition Histoplasma reactivation can occur in immune-suppressed residents of northern European countries years after leaving histoplasma capsulatum endemic areas such as South America.
REFERENCES


Haematemesis, abdominal pain and a diastolic murmur in a cocaine user

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CASE REPORT

A 39-year-old man was admitted to our hospital with haematemesis. After the use of cocaine earlier that same evening he experienced a tingling sensation, followed by haematemesis and upper abdominal pain. His medical history revealed cocaine and heroin abuse. He was not on any medication.

On physical examination the patient appeared to be alert and in no acute distress. His blood pressure was 115/35 mmHg, the pulse rate was 76 beats/min. Except for mild epigastric tenderness, no abnormalities were found. The electrocardiogram (ECG) showed sinus rhythm with nonspecific repolarisation changes. Routine laboratory tests and chest X-ray were normal.

The patient was admitted to medium care for observation of haematemesis in suspected upper gastrointestinal bleeding.

During the night the patient experienced increasing abdominal pain. Additionally he started to complain of back pain. Therefore the patient was reassessed. Blood pressure was 120/25 mmHg, in both arms. Auscultation now revealed a diastolic murmur in the left second intercostal space. His abdomen was more painful, with rebound tenderness. An emergency computed tomography angiography (CT-a) was performed (figure 1).

WHAT IS YOUR DIAGNOSIS?

See page 426 for the answer to this photo quiz
A 46-year-old man presented in our outpatient HIV clinic with a seven-day history of a mildly itchy rash without other complaints. He had been HIV positive since 2003. Furthermore, his history included chronic hepatitis C (genotype 1b) and a scabies infection in 2007. He was not on any medication at the time of presentation. He had not travelled recently. On examination, we found a maculopapular rash (figure 1) on the whole body, including his face. His temperature was 37 °C. Further physical examination was unremarkable. His laboratory tests showed a CD4⁺ lymphocyte count of 150 × 10⁶/l and slightly elevated liver enzymes, with an alanine aminotransferase level of 50 U/l and an aspartate aminotransferase level of 63 U/l.

WHAT IS YOUR DIAGNOSIS?

See page 428 for the answer to this photo quiz.
A 60-year-old woman presented with hoarseness, dyspnoea, dysphagia, and anterior neck discomfort. Physical examination revealed an enlarged thyroid with substernal extension. A computed tomography (CT) scan of the neck and chest revealed diffuse homogeneous enlargement of both the thyroid lobes extending to the mediastinum and associated with tracheal compression (Figure 1). No cervical or mediastinal lymphadenopathy was detected. The patient was in euthyroid state biochemically and clinically.

The patient's compressive symptoms resolved after performing a total thyroidectomy. Pathology revealed evidence of fibrosis surrounding small nodules of residual follicles with massive infiltration by plasma cells (Figure 2).

**WHAT IS YOUR DIAGNOSIS?**

See page 429 for the answer to this photo quiz.
PHOTO QUIZ

Solid as a rock?

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SUMMARY

A short report on bilateral breast calcifications in a patient with renal failure.

CASE REPORT

A 68-year-old woman was referred by the internist to the radiology department for screening for carcinoma. Her medical history showed peripheral artery thrombosis in both legs, essential hypertension, diabetes mellitus type 2, chronic renal failure (GFR 15 ml/min), and several abdominal operations (for non-malignant disease). She had lost 10 kg bodyweight in several months (current weight: 40 kg). An X-ray of the lungs was performed. Multiple, bilateral calcifications were seen in the breasts and a mammography was performed (figures 1 to 4).

WHAT IS YOUR DIAGNOSIS?

See page 430 for the answer to this photo quiz.

Figures 1 and 2. Chest X-ray. Focussing on the lower part of the image; there are numerous calcifications in the soft tissues. The lateral view depicts the calcifications more clearly in the breasts.

Figures 3 and 4. Mammogram. Extensive heterogeneous, coarse, part lobulated calcifications scattered throughout both breasts.
Diagnosis

The presence of haematemesis and abdominal pain suggested acute abdominal disease. However, the combination of severe pain and signs of aortic insufficiency, namely diastolic murmur and high pulse pressure, made us think of aortic dissection. CT-a indeed showed a Stanford type-A aortic dissection. The patient was immediately transferred to a cardiac surgery centre and a supracoronary aorta ascendens replacement was performed. The haematemesis resolved spontaneously and did not reoccur. No explanation was found as the CT scan showed neither perforation of the aorta into the oesophagus, nor signs of intestinal ischaemia. The patient recovered.

The clinical presentation of acute aortic dissection (AAD) can be atypical and often mimics other diseases.1,2 This leads to a diagnostic delay in 39 to 85% of cases, thereby increasing mortality and morbidity.1,2 Initially, 32% of AADs are mistaken for acute coronary syndrome.2 Without treatment, mortality of AAD is about 40 to 50% in the first 48 hours.1,4 Therefore, it is vital to recognise risk factors and symptoms (table 1). The most important risk factors are hypertension and male gender.3 Cocaine abuse, as in our patient, is increasingly reported as risk factor.3-5 The presenting symptoms are the result of local complications caused by the dissection.1,3 The dissection flap can cause occlusion of a branch artery resulting in ischaemia of heart, brain, kidney, spinal cord, intestines and/or extremities.1,5 If the tear communicates with the pericardium, pericardial tamponade results.1 Pain, usually very severe, is the most common presenting symptom (90%).1,3 Importantly, in 10% of the cases AAD is painless.3 Pulse deficit is present in less than half of the patients.3,5 Diastolic murmur, a sign of aortic insufficiency, is usually present in type A dissection. Haematemesis can occur as a result of intestinal ischaemia or perforation to the oesophagus. Most patients have abnormal chest radiography and ECG.1,3 AAD should always be considered in patients presenting with unexplained chest, back or abdominal pain, neurological deficit, syncope, pulse deficit, diastolic murmur, high pulse pressure, kidney failure and/or ischaemia of limbs or intestines.

Acknowledgements

We thank R.H. Kruyt, radiologist, for providing us with the CT-angiography image.

Table 1. Acute aortic dissection: associated factors, presenting features and findings in routine tests

<table>
<thead>
<tr>
<th>Associated factors</th>
<th>Type A (n=67)</th>
<th>Type B (n=384)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>61</td>
<td>65</td>
</tr>
<tr>
<td>Male sex</td>
<td>67%</td>
<td>71%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>67%</td>
<td>86%</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>28%</td>
<td>38%</td>
</tr>
<tr>
<td>Previous cardiovascular surgery</td>
<td>16%</td>
<td>17%</td>
</tr>
<tr>
<td>Aortic aneurysm</td>
<td>7%</td>
<td>18%</td>
</tr>
<tr>
<td>Marfan’s syndrome</td>
<td>6%</td>
<td>3%</td>
</tr>
<tr>
<td>Related to coronary angiography</td>
<td>6%</td>
<td>2%</td>
</tr>
<tr>
<td>Bicuspid aortic valve</td>
<td>4%</td>
<td>2%</td>
</tr>
<tr>
<td>Previous aortic dissection</td>
<td>3%</td>
<td>9%</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Cocaine abuse</td>
<td>&lt;1%</td>
<td>1%</td>
</tr>
</tbody>
</table>

Symptoms and signs

Pain
- Chest or back pain 83% 86%
- Abdominal pain 22% 43%
- Severe or worst-ever pain 90% 90%
- Abrupt onset of pain 91% 89%
- Migrating pain 13% 25%
- Hypotension, shock or tamponade 27% 3%
- Hypertension 36% 69%
- Any pulse deficit 31% 21%
- Aortic regurgitation 44% 12%
- Focal neurological deficit 17% 5%

Chest x-ray
- Normal 11% 21%
- Widened mediastinum 63% 56%
- Abnormal aortic contour 47% 49%

ECG
- Normal 30% 31%
- Left ventricular hypertrophy 23% 32%
- Myocardial ischaemia or infarction 24% 10%

The classical triad
- Aortic pain + pulse deficit + widened mediastinum
- Triad present in 27% of patients
- Presence of triad has a positive likelihood ratio of 66.0

Data as published by the International Registry of Acute Aortic Dissection (IRAD).4,5 Vascular surgery includes coronary artery bypass surgery, aortic valve replacement, aortic aneurysm or dissection repair, mitral-valve replacement of repair, or other aortic surgery. 6 Klompas identified significant negative and positive indicators for AAD. The presence of pulse deficit or focal neurological deficit increases the probability of AAD. The classical triad of aortic pain, pulse deficit and mediastinal widening is a strong indicator of AAD. Negative indicators are absence of acute pain, normal chest X-ray, absence of symptoms from the triad.
REFERENCES


**DIAGNOSIS**

Although our patient had no history of a chancre, the typical maculopapular rash, in a patient known with HIV, raised the suspicion of syphilis. This diagnosis was confirmed by a rapid plasma reagin (RPR) titre of 1:128 and a *Treponema pallidum* haemagglutination assay (TPHA) titre of 1: >20,480, which were negative and 1: 320, respectively, three months earlier. To examine whether there was any central nervous system (CNS) involvement a lumbar puncture was performed which showed 30 leukocytes/µl, a total protein level of 0.54 g/l and normal glucose levels. RPR was negative and the TPHA titre was 1:80. Our patient was successfully treated for (neuro) syphilis with four million units of intravenous penicillin every four hours for ten days. Serological titres decreased over time.

There seems to be an increased rate of early (asymptomatic) neurosyphilis among HIV-infected patients, which might be due to insufficient control of the infection. Case reports of HIV patients who developed neurosyphilis despite adequate intramuscular treatment of early syphilis prompted many physicians to perform routine lumbar punctures in all coinfected patients. However, diagnosing neurosyphilis in HIV patients is difficult since pleocytosis and elevated cerebrospinal fluid (CSF) protein levels are common findings in HIV-infected patients. Furthermore, RPR testing has a high specificity but a low sensitivity. TPHA testing is very useful when it is negative but a positive titre can falsely imply CNS involvement, since antibodies can cross the blood-brain barrier. Therefore, deciding who should undergo a lumbar puncture is currently one of the most controversial issues in the management of coinfected patients. The Centers for Disease Control and Prevention and most experts now agree that CSF examination must be performed in HIV-infected patients who have late latent syphilis, syphilis of unknown duration, neurological signs or symptoms, or suspected treatment failure. Additionally, recent studies found an association between neurosyphilis and RPR titres of >1:32 and CD4+ lymphocyte count of <350 × 10^6/l, so CSF examination should be considered in these cases. Further studies are warranted to establish which coinfected patients are most likely to benefit from CSF examination.

**REFERENCES**


Diagnosis: Plasmacytoma in thyroid

The massive infiltration of the thyroid by plasma cells is consistent with thyroid plasmacytoma. The plasma cell infiltrate was mostly of the kappa type. Immunoperoxidase stains for polyclonal kappa and lambda revealed cytoplasmic staining for kappa in 99% of the cells with staining for lambda in less than 1%. Serum protein electrophoresis showed a mildly elevated gamma globulin band of 1.8 g/dl (range: 0.5 to 1.5 g/dl) and a postoperative radioiodine scan was negative for any evidence of residual thyroid tissue.

Plasmacytoma is a rare tumour of the thyroid gland with only 46 cases reported. The head and neck are the most common sites of isolated extramedullary plasmacytoma, whereas liver, spleen, and lymph nodes are the most frequent extramedullary sites of involvement in systemic multiple myeloma. Extramedullary plasmacytoma may be a primary isolated lesion with or without affected lymph nodes or an extramedullary manifestation of systemic multiple myeloma. CT and magnetic resonance imaging are the imaging methods of choice for demonstrating extramedullary manifestations of systemic or isolated plasmacytoma. CT and ultrasound are both useful in guiding biopsies. Although, fine needle aspiration (FNA) has been widely used in the diagnosis of nodular thyroid disorders, there have been limited experiences with preoperative diagnosis of thyroid plasmacytomas. With FNA, a thyroid plasmacytoma can be mistaken for thyroid lymphoma and even medullary carcinoma.

Plasmacytoma can be treated non-surgically using thalidomide-dexamethasone combination treatment or radiation therapy. Plasmacytoma without medullary lesions has a favourable prognosis (15 years survival rate of 78%) when treated locally by irradiation and/or surgery. Surgical intervention is safe and radiation therapy should be considered in patients with soft tissue and long bone metastases, due to the associated complications. Long-term follow-up is recommended to monitor for possible progression to multiple myeloma.

References

DISCUSSION

Widespread calcifications of the breast are commonly found in patients after trauma (haematoma), in fat necrosis, with breast prosthesis, and after surgery and radiotherapy. In patients with end-stage renal disease (ESRD), calcium deposits in the soft tissues are also a known entity. High serum calcium-phosphate (due to hyperparathyroidism), as well as uraemia, increased parathyroid hormone levels, excess of vitamin D and the presence of local tissue injury, are described as predisposing factors for calcium deposits all over the soft tissues. Most common sites of deposition are the blood vessels, cornea, peri-articular tissues, skin and visceral organs. Extensive calcifications in the breasts are rare. In our patient calcifications were only identified in the breasts. These calcifications may vary in time, in number and aspect in patients with renal failure, (reducing with decreasing phosphate levels). Restriction of dietary phosphate, administrating phosphate-binding gels to prevent absorption of ingested phosphate and haemodialysis (decrease of serum phosphate levels) have been described to reduce soft tissue calcifications.

Breast cancer is the single most common malignancy in women. The standard method of imaging in screening for breast cancer in the Netherlands is a mammogram, with or without additional ultrasound. On mammogram, one of the presentations of cancer is (clustered) calcifications. Breast calcifications in general are classified into four groups; vascular, parenchymal, ductal and miscellaneous. Vascular and parenchymal calcifications can occur in ESRD. In women with ESRD and calcium deposits in the breasts, this can provide a diagnostic challenge in the detection of breast cancer.

REFERENCES

Quantifying exposure to calcium and phosphate in ESRD: predictive of atherosclerosis on top of arteriosclerosis?

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ABSTRACT

Background: Long-term exposure to hypercalcaemia and hyperphosphataemia leads to media calcification and predicts mortality in patients with end-stage renal disease (ESRD). It is debatable whether this exposure is only a risk factor for arteriosclerosis, or also for superimposed atherosclerosis. Calcium-phosphate exposure is difficult to quantify, because it is variable in time and exerts its deleterious effects only after prolonged presence.

Methods: In 90 dialysis patients, calcium and phosphate values from the complete dialysis period were collected. From three-month averages, measures for calcium-phosphate exposure were derived after exclusion of transplant periods. Calcium-phosphate exposure was then related to intima-media thickness (IMT) and to ankle-brachial index (ABI) as markers of early atherosclerosis.

Results: Calcium-phosphate exposure was quantified in three ways using 1670 patient-quarters (i.e. three-months periods) covering 93% of the time on dialysis: averaged calcium-phosphate exposure, percentage of time with above-reference values, and burden of hypercalcaemia/hyperphosphataemia represented by this percentage multiplied by months on dialysis. No association was found with IMT. Patients with increased, not decreased, ABI had higher calcium-phosphate exposure throughout dialysis treatment: hyperphosphataemia burden was 31 (19 to 43) months for patients with ABI between 0.90 and 1.40 and 79 (58 to 100) months for patients with ABI >1.40 or incompressible ankle arteries (p<0.001).

Conclusion: These findings do not support the hypothesis that calcium-phosphate exposure leads to atherosclerotic changes on top of arteriosclerosis in ESRD, and confirm its role in causing arteriosclerotic damage leading to increased arterial stiffness and incompressible ankle arteries. The used tool for quantifying calcium-phosphate exposure is easy to apply and can properly weigh the complete exposure during ESRD.

KEYWORDS

Atherosclerosis, calcium, haemodialysis, phosphate, quantification

INTRODUCTION

Cardiovascular disease is the leading cause of mortality in patients with end-stage renal disease (ESRD). Long-term exposure to increased concentrations of calcium and phosphate is an important predictor of mortality in these patients, and is progressively seen as the main focus of therapy.1-6 This exposure is difficult to quantify because it is variable in time and exerts its deleterious effects only after prolonged presence.

There is an ongoing debate about the predominance of atherosclerotic versus arteriosclerotic abnormalities in ESRD.7-9 Atherosclerosis is characterised by plaque-forming degenerative intima changes of the aorta and of large elastic arteries resulting in vessel obstruction, whereas in arteriosclerosis there is concentric media...
thickening of muscular arteries primarily leading to vascular stiffening. Both processes can be accompanied with calcification, but with a distinct pattern. Intima calcification is characterised by patchy calcification of the intima around lipid deposits as present in plaque calcification, considered as classical atherosclerosis, and ascribed to hyperlipidaemia and age-related degeneration. Media calcification is characterised by absence of lipid deposits, but by metabolite-induced vascular changes that lead to upregulation of osteogenic differentiation of vascular smooth muscle cells; it is primarily attributed to increased calcium-phosphate levels. This process is typical for ESRD, as it can be induced in animal models of chronic kidney disease and already occurs in young adults with ESRD. Even with advanced imaging techniques, the distinction between intimal and medial calcification is difficult. Still, because in ESRD the pathophysiology, prognosis and treatment differ for atherosclerosis and arteriosclerosis, it may be important to distinguish these processes when possible.

We had a population of patients with ESRD, for whom ABI measurements according to the study protocol. Of a terminal malignancy. All patients underwent IMT and ABI measurements. Entry criteria for the present study were: treatment with chronic outpatient peritoneal dialysis or haemodialysis, age between 18 and 80 years and absence of a terminal malignancy. All patients underwent IMT and ABI measurements according to the study protocol.

The study was approved by the ethics committee of the University Medical Center Utrecht and written informed consent was obtained from all participants.

**Data collection and calcium-phosphate monitoring**

Data regarding patient demographics, medical history and laboratory examinations including risk factors for atherosclerosis were collected from self-report questionnaires and chart reviews, at the time of the IMT and ABI measurements.

During the years of dialysis treatment, phosphate binder prescription was aimed at a phosphate <1.7 mmol/l and a calcium <2.60 mmol/l, with the use of various phosphate binders including calcium carbonate and calcium acetate, aluminium hydroxide in early years and sevelamer in recent years.

Calcium-phosphate exposure was assessed by collecting all available calcium and phosphate values, generally measured twice a month, from the start of renal replacement therapy until the IMT/ABI measurements. If a patient had had a functioning renal transplant between the start of renal replacement therapy and the IMT/ABI measurements, values from the transplant period were excluded. All available calcium and phosphate values were averaged for every three months (quarter) of dialysis of a given patient; the calcium-phosphate product (Ca*P) was also averaged per quarter. From these quarterly averages, the following three measures for calcium-phosphate exposure were derived:

A. Averaged values for calcium, phosphate and Ca*P for the complete dialysis period;

B. Percentage of time on dialysis with above-reference levels, i.e. the number of dialysis-quarters a patient had with mean calcium >2.60 mmol/l, phosphate >1.70 mmol/l or Ca*P >4.50 mmol²/l², divided by the total number of quarters this patient was treated with dialysis, multiplied by 100%.

C. Cumulative burden of hypercalcaemia and hyperphosphataemia, i.e. the percentage of dialysis time a patient had calcium >2.60 mmol/l, phosphate >1.70 mmol/l or Ca*P >4.50 mmol²/l², multiplied by the total duration of dialysis in months of this patient; this burden represents the absolute number of months on dialysis a patient had above-reference levels of calcium and phosphate.

**Materials and Methods**

**Study design and research population**

The present study is a prospective cohort study of all haemodialysis and peritoneal dialysis patients who participated in the Second Manifestations of ARTerial disease (SMART) study. SMART is an ongoing prospective study of patients with manifestations of or risk factors for vascular disease. Entry criteria for the present study were: treatment with chronic outpatient peritoneal dialysis or haemodialysis, age between 18 and 80 years and absence of a terminal malignancy. All patients underwent IMT and ABI measurements according to the study protocol.

**Carotid artery intima-media thickness and ankle-brachial index**

Left and right common carotid arteries were examined in the anterolateral, posterolateral, and mediolateral directions with an ATL Ultramark 9 (Advanced Technology Laboratories) equipped with a 10-MHz linear-array transducer, as described previously. The mean IMT of six measurements in each patient was calculated, and categorised in tertiles. Plaques and stenosis of the common
and internal carotid arteries at both sides were measured with colour Doppler-assisted Duplex scanning.

The ABI was obtained by computing the ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm. In haemodialysis patients, blood pressure was obtained from the arm without fistula or graft. For the analyses, we used the values of both legs, and categorised these as ≤0.90, >0.90 and ≤1.40, or >1.40. In case of discrepancy in category between the legs, the patient was categorised according to the most abnormal leg. After leg amputation, ABI was categorised as ≤0.90; when arterial compression was not possible due to arterial rigidity, ABI was categorised as >1.40.

Statistical analysis
ANOVA with generalised linear models were used to compare unadjusted and adjusted means of the calcium-phosphate parameters for each category of IMT and ABI. Averaged values for calcium and phosphate were adjusted for age, sex and duration of dialysis treatment. The analyses of the other two measures of calcium-phosphate exposure were only adjusted for age and sex, in order to do justice to the long-standing exposure to calcium and phosphate.

Statistical analyses were performed using SAS (vs. 8.2, SAS institute Inc, Cary, North Carolina, United States) and SPSS software (vs. 15.0; SPSS Inc. Headquarters, Chicago, Illinois, United States).

RESULTS

The SMART study included 90 patients with ESRD; characteristics are listed in table 1. Seventy-six patients were on haemodialysis and 14 on peritoneal dialysis at the time of the IMT/ABI studies. Median duration of renal replacement therapy was 39 months (interquartile range (IQR) 16 to 104). Twenty-three patients had had a temporarily functioning kidney transplant for 43 months (IQR 24 to 74).

In table 2, the calcium and phosphate exposure during the complete period of dialysis treatment is presented. There were no differences in exposure between patients treated with haemodialysis compared with patients treated with peritoneal dialysis. Averaged calcium concentration was 2.46±0.15 mmol/l, averaged phosphate 1.83±0.38 mmol/l and averaged Ca*P was 4.50±0.90 mmol2/l2. In total, 1670 quarters with calcium-phosphate data were collected, covering 93% of the time patients were treated with a form of dialysis.

### Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>ESRD (n=90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
</tr>
<tr>
<td>Age at baseline (years)*</td>
<td>51.6±12.8</td>
</tr>
<tr>
<td>Men</td>
<td>72%</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>22%</td>
</tr>
<tr>
<td>Ever smoking</td>
<td>77%</td>
</tr>
<tr>
<td>Duration of dialysis (months)*</td>
<td>39 (16, 104)</td>
</tr>
<tr>
<td>Renal transplant in past</td>
<td>26%</td>
</tr>
<tr>
<td>Localisation of cardiovascular disease</td>
<td></td>
</tr>
<tr>
<td>Cerebrovascular disease in past</td>
<td>2%</td>
</tr>
<tr>
<td>Coronary arterial disease in past</td>
<td>13%</td>
</tr>
<tr>
<td>Aortic aneurysm in past</td>
<td>2%</td>
</tr>
<tr>
<td>Peripheral arterial disease in past</td>
<td>7%</td>
</tr>
<tr>
<td>Modifiable risk factors</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)#</td>
<td>23.8±3.7</td>
</tr>
<tr>
<td>Waist-hip ratio#</td>
<td>0.9±0.09</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)#</td>
<td>119±21</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)#</td>
<td>80±11</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)#</td>
<td>79±18</td>
</tr>
<tr>
<td>Serum measurements</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)#</td>
<td>4.5±1.1</td>
</tr>
<tr>
<td>Fasting triglycerides (mmol/l)#</td>
<td>2.2±1.6</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)#</td>
<td>1.2±0.4</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)#</td>
<td>2.3±0.9</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)#</td>
<td>10.9±14.1</td>
</tr>
<tr>
<td>Albumin (g/l)#</td>
<td>39.9±22.8</td>
</tr>
<tr>
<td>Parathyroid hormone (pmol/l)#</td>
<td>30.4±31.4</td>
</tr>
</tbody>
</table>

*median (IQR 25%, 75%) # = mean ± SD

<table>
<thead>
<tr>
<th></th>
<th>ESRD (n=90)</th>
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</thead>
<tbody>
<tr>
<td>Averaged serum calcium (mmol/l)</td>
<td>2.46±0.15</td>
</tr>
<tr>
<td>Averaged serum phosphate (mmol/l)</td>
<td>1.83±0.38</td>
</tr>
<tr>
<td>Averaged Ca*P product (mmol2/l2)</td>
<td>4.50±0.90</td>
</tr>
<tr>
<td>Percentage time on dialysis with calcium ≥2.60 mmol/l (%)#</td>
<td>29±28</td>
</tr>
<tr>
<td>Percentage time on dialysis with phosphate ≥1.70 mmol/l (%)#</td>
<td>61±43</td>
</tr>
<tr>
<td>Percentage time on dialysis with Ca*P product ≥2.45 mmol2/l2 (%)#</td>
<td>52±33</td>
</tr>
<tr>
<td>Hypercalcaemia burden* (months)</td>
<td>29±45</td>
</tr>
<tr>
<td>Hyperphosphataemia burden* (months)</td>
<td>46±43</td>
</tr>
<tr>
<td>Hypercalcaemia/hyperphosphataemia burden* (months)</td>
<td>39±51</td>
</tr>
</tbody>
</table>

*percentage of time on dialysis with above-reference values, i.e. the number of dialysis-quarters (3-month periods) a patient had with mean calcium, phosphate or Ca*P above the given value, divided by the number of total quarters this patient was treated with dialysis, multiplied by 100%

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<th>ESRD (n=90)</th>
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*cumulative burden of hypercalcaemia and hyperphosphataemia, i.e. the percentage of dialysis time a patient had with calcium ≥2.60 mmol/l, phosphate ≥1.70 mmol/l or Ca*P ≥4.50 mmol2/l2, multiplied by the total duration of dialysis in months for this patient. All results in above table are presented by mean ± SD.

### Table 2. Parameters of calcium-phosphate exposure during the complete period of dialysis treatment

Van Jaarsveld, et al. Calcium-phosphate exposure in ESRD.
of cholesterol, C-reactive protein (CRP), albumin and parathyroid hormone (PTH) were comparable between the IMT groups.

Table 3 provides the data concerning calcium and phosphate exposure for patients in the different IMT tertiles. For A: averaged values, B: percentage of time with above-reference values, and C: burden of hypercalcaemia/ hyperphosphataemia, no differences were found between the IMT groups in the unadjusted analyses, nor were differences present after adjustment for age, sex and duration of dialysis, where applicable.

Ankle-brachial index
Mean ABI was 1.22±0.21 in 86 patients. Six patients had an ABI of less than 0.90, including one with an amputation. Of the 19 patients in the category ABI >1.40, eight had non-compressible ankle arteries, resulting in an infinitely high ABI. The patients in the lowest ABI category had an ABI of less than 0.90, including one with an

Table 3. Calcium and phosphate parameters for different categories of IMT

<table>
<thead>
<tr>
<th>IMT</th>
<th>Averaged calcium (mmol/l)</th>
<th>Averaged phosphate (mmol/l)</th>
<th>Averaged Ca-P product (mmol²/l²)</th>
<th>Percentage time on dialysis with calcium ≥2.60 mmol/l (%)</th>
<th>Percentage time on dialysis with phosphate ≥1.70 mmol/l (%)</th>
<th>Hypercalcaemia burden (months)</th>
<th>Hyperphosphataemia burden (months)</th>
<th>Hypercalcaemia/hyperphosphataemia burden (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lowest tertile</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>2.49 (2.43-2.55)</td>
<td>1.16 (1.11-1.21)</td>
<td>2.71 (2.65-2.78)</td>
<td>0.70 (0.63-0.75)</td>
<td>0.69 (0.62-0.76)</td>
<td>0.98 (0.89-1.07)</td>
<td>0.96 (0.88-1.04)</td>
<td>0.76 (0.68-0.84)</td>
</tr>
<tr>
<td>Adjusted**</td>
<td>2.51 (2.45-2.58)</td>
<td>1.16 (1.11-1.21)</td>
<td>2.74 (2.68-2.80)</td>
<td>0.70 (0.63-0.75)</td>
<td>0.69 (0.62-0.76)</td>
<td>0.98 (0.89-1.07)</td>
<td>0.96 (0.88-1.04)</td>
<td>0.76 (0.68-0.84)</td>
</tr>
<tr>
<td>Intermediate tertile</td>
<td>2.44 (2.39-2.50)</td>
<td>1.16 (1.11-1.21)</td>
<td>2.71 (2.65-2.78)</td>
<td>0.70 (0.63-0.75)</td>
<td>0.69 (0.62-0.76)</td>
<td>0.98 (0.89-1.07)</td>
<td>0.96 (0.88-1.04)</td>
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<td>Unadjusted</td>
<td>2.44 (2.39-2.50)</td>
<td>1.16 (1.11-1.21)</td>
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<td>Adjusted**</td>
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<tr>
<td>Highest tertile</td>
<td>2.47 (2.41-2.52)</td>
<td>1.16 (1.11-1.21)</td>
<td>2.74 (2.68-2.80)</td>
<td>0.70 (0.63-0.75)</td>
<td>0.69 (0.62-0.76)</td>
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<td>0.76 (0.68-0.84)</td>
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</table>

*adjusted for age, sex and duration of dialysis treatment; **adjusted for age and sex; †percentage of time on dialysis with above-reference values, i.e. the number of dialysis quarters (3-month periods) a patient had with mean calcium, phosphate or Ca*P above the given value, divided by the total number of quarters this patient was treated with dialysis, multiplied by 100%; *cumulative burden of hypercalcaemia and hyperphosphataemia, i.e. the percentage of dialysis time a patient had with calcium ≥2.60 mmol/l, phosphate ≥1.70 mmol/l or Ca*P ≥4.50 mmol²/l², multiplied by the total duration of dialysis in months of this patient.

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adjustment for time on dialysis, but only for hyperphosphataemia and combined hypercalcaemia/hyperphosphataemia: adjusted hypercalcaemia burden was 27 months (95% CI 20 to 33) for the middle ABI and 26 months (95% CI 14 to 39) for the high ABI group (p=0.97), adjusted hyperphosphataemia burden was 38 (95% CI 31 to 44) vs 56 months (95% CI 44 to 68, p=0.01) and combined hypercalcaemia/hyperphosphataemia burden was 32 (95% CI 26 to 37) vs 49 months (95% CI 39 to 60) for the respective groups (p=0.006).

**Discussion**

After longitudinally collecting all calcium and phosphate values from the patients’ complete periods of dialysis, the associations between several parameters of calcium and phosphate exposure and the early markers of atherosclerosis IMT and ABI were examined. We found that calcium-phosphate exposure was not correlated with IMT, irrespective of which parameter of exposure was chosen. Regarding ABI, high calcium-phosphate exposure did not predict a decreased ABI, the established marker of atherosclerosis, but was instead predictive of increased ABI.

Intima-media thickness is a generally applied marker of atherosclerosis. An increased IMT was not mediated by calcium-phosphate exposure, and no trend was present suggesting any relation between calcium-phosphate exposure and IMT. Literature data show conflicting results on this issue. Phosphate, but not calcium, was found to be associated with increased IMT in haemodialysis patients. 15 In studies of IMT and carotid plaque formation, calcium was associated with plaque formation but not with IMT, 16 and plaque formation but not IMT, was associated with cardiovascular events. 17 Furthermore, it seems that in dialysis patients IMT values are difficult to interpret, because the homogeneity of the carotid intima-media is disturbed. 18 However, in all of these studies either once-measured calcium and phosphate 16,17 or values from three to six months of dialysis 15,18 were used, whereas the present study used the exposure during the complete dialysis period. To cope with the skewed distribution of IMT and the difficulty in distinguishing patients with

<table>
<thead>
<tr>
<th>Table 4. Calcium and phosphate parameters for different categories of ABI</th>
<th>ABI</th>
<th>ABI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.90 and ≤1.40</td>
<td>&gt;1.40</td>
<td>n=61</td>
</tr>
<tr>
<td><strong>Averaged serum calcium (mmol/l)</strong></td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>2.46 (2.42-2.50)</td>
<td>2.51 (2.44-2.58)</td>
</tr>
<tr>
<td>Adjusted*</td>
<td>2.46 (2.42-2.50)</td>
<td>2.51 (2.44-2.58)</td>
</tr>
<tr>
<td><strong>Averaged serum phosphate (mmol/l)</strong></td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.81 (1.73-1.92)</td>
<td>1.91 (1.75-2.08)</td>
</tr>
<tr>
<td>Adjusted*</td>
<td>1.82 (1.72-1.91)</td>
<td>1.94 (1.76-2.11)</td>
</tr>
<tr>
<td><strong>Averaged Ca-P product (mmol²/l²)</strong></td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>4.48 (4.26-4.70)</td>
<td>4.76 (4.37-5.13)</td>
</tr>
<tr>
<td>Adjusted*</td>
<td>4.46 (4.24-4.69)</td>
<td>4.81 (4.39-5.23)</td>
</tr>
<tr>
<td><strong>Percentage time on dialysis with calcium ≥2.60 mmol/l (%)</strong>*</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>28 (20-35)</td>
<td>36 (23-49)</td>
</tr>
<tr>
<td>Adjusted**</td>
<td>27 (20-34)</td>
<td>37 (24-50)</td>
</tr>
<tr>
<td><strong>Percentage time on dialysis with phosphate ≥1.70 mmol/l (%)</strong>*</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>63 (55-71)</td>
<td>62 (48-77)</td>
</tr>
<tr>
<td>Adjusted**</td>
<td>63 (54-71)</td>
<td>63 (48-78)</td>
</tr>
<tr>
<td><strong>Percentage time on dialysis with Ca-P product ≥4.50 mmol²/l² (%)</strong>†</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>52 (44-61)</td>
<td>54 (39-69)</td>
</tr>
<tr>
<td>Adjusted**</td>
<td>52 (43-60)</td>
<td>55 (40-70)</td>
</tr>
<tr>
<td><strong>Hypercalcaemia burden (months)</strong>*</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>19 (7-31)</td>
<td>50 (28-72)</td>
</tr>
<tr>
<td>Adjusted**</td>
<td>19 (7-31)</td>
<td>51 (28-73)</td>
</tr>
<tr>
<td><strong>Hyperphosphataemia burden (months)</strong>†</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>30 (19-42)</td>
<td>80 (59-101)</td>
</tr>
<tr>
<td>Adjusted**</td>
<td>31 (19-43)</td>
<td>79 (58-100)</td>
</tr>
<tr>
<td><strong>Hypercalcaemia/hyperphosphataemia burden (months)</strong>#</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>25 (14-35)</td>
<td>71 (52-90)</td>
</tr>
<tr>
<td>Adjusted**</td>
<td>25 (15-36)</td>
<td>70 (51-89)</td>
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</table>

*adjusted for age, sex and duration of dialysis treatment; **adjusted for age and sex; †percentage of time on dialysis with above-reference values, i.e. the number of dialysis quarters (3-month periods) a patient had with mean calcium, phosphate or Ca*P above the given value, divided by the total number of quarters this patient was treated with dialysis, multiplied by 100%; #cumulative burden of hypercalcaemia and hyperphosphataemia, i.e. the percentage of dialysis time a patient had with calcium ≥2.60 mmol/l, phosphate ≥1.70 mmol/l or Ca*P ≥4.50 mmol²/l², multiplied by the total duration of dialysis in months for this patient.

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high IMT from patients with early plaque formation, we chose to divide the patients in tertiles of IMT. Hence the patients with very high IMT and/or carotid plaques were included in the high IMT group. Nevertheless, when the analyses were done with IMT as continuous variable, or after exclusion of patients with IMT values above 1.20, the results were virtually the same. We conclude that although increased IMT is an established risk factor for mortality in general, it is not influenced by calcium-phosphate exposure in our patients. This part of the data therefore rejects the hypothesis that calcium-phosphate exposure contributes to early atherosclerosis in ESRD. This result is supported by Bui et al. who recently found no relation between severity of kidney dysfunction and carotid IMT, also suggesting that classical atherosclerosis plays a minor role in the increased cardiovascular risk in renal disease.29

How to interpret the association between calcium-phosphate exposure and not decreased, but increased ABI? ABI, the other marker for atherosclerosis in this study, is also a known risk factor for mortality in patients with ESRD. Remarkably, a decreased as well as an increased ABI were reported to predict increased mortality rates.30-32 It is presumed that low ABI reflects generalised atherosclerosis, whereas high ABI reflects media calcification and stiffened vessels.32,34,36 Surprisingly, very few patients appeared to have a decreased ABI in our study despite high calcium-phosphate exposure; this could be the result of inclusion bias, or reflect a low rate of obstructed peripheral arteries by classical atherosclerosis. There was a clear association between calcium-phosphate exposure and increased ABI, in particular for cumulative burden of hypercalcaemia and hyperphosphataemia (table 4). Literature data on this issue are scarce. A small study on the relation between left ventricular mass and ABI found a reverse correlation between ABI and once-measured calcium and phosphate.37 More convincing data from the Multi-Ethnic Study of Atherosclerosis (MESA) cohort showed a strong association between quartiles of phosphate concentrations and high ABI, but again phosphate was measured only once at the time of the ABI study.38 Our study confirms and extends the latter study in finding the same results while using complete calcium-phosphate exposure. It was specifically the total burden of hyperphosphataemia and combined hyperphosphataemia/hypercalcaemia, i.e. the absolute time period a patient had the above-reference levels, which predicted a high ABI. This is the first study finding a relation between high ABI and time-averaged measures of calcium-phosphate exposure. It fits in with the hypothesis that the calcification process only takes place in periods when calcium and phosphate exceed their solubility product, possibly in combination with shortage of calcification inhibitor proteins.39 It also underscores the findings of the group of London et al. of increased arterial stiffening after longstanding dialysis,40-43 and again hypothesises that atherosclerosis probably plays a minor role. To prove that not the duration of dialysis per se could explain the results, the additional adjustment for time on dialysis was done, after which not hypercalcaemia burden, but still the burden of high phosphate and high calcium-phosphate product remained significant.

In this study, a new method to quantify exposure to high calcium and phosphate levels is presented. Most studies so far report on calcium and phosphate measured at one moment in time, or sometimes use averaged values for three to six months of dialysis.38,39,41 However, in many patients treated with dialysis, there are prolonged periods with either low or high exposure. As hypercalcaemia and hyperphosphataemia are such important risk factors for morbidity and mortality in ESRD, and are frequently monitored in clinical practice, it is rather unsatisfying to use so little of the available calcium and phosphate information for risk stratification. Furthermore, hypercalcaemia and hyperphosphataemia exert their unfavourable influence only after prolonged periods of time. A method to determine the presence of these risk factors in an objective and reproducible way is therefore essential.

Calculation of quarterly averages of serum calcium and phosphate enabled inclusion of all measurements, and periods with frequent measurements could be equally weighed as periods with scarce measurements. Calcium-phosphate data from >90% of dialysis time of the patients were collected, covering more than 400 patient-years. These quarterly averages enabled subsequent calculation of the three measures of calcium-phosphate exposure, representing (A) overall exposure, (B) the percentage of time on dialysis in which calcium and phosphate were not well controlled, and (C) the absolute length of time with above-reference values. By using these measures, short periods of time with high exposure in a patient can be identified, even if average levels are not increased.

The choice of the reference values is arbitrary. Most of the data used for this study result from a period before the strict Kidney Disease Outcomes Quality Initiative (KDOQI) Guideline for Bone Metabolism and Disease was applied. calcium was targeted below 2.60 mmol/l in this period, and phosphate below 1.70 mmol/l, which is why these values were chosen as cut-off levels. The phosphate concentration averaged over the complete duration of dialysis was of the same order as other cross-sectional1 or time-averaged data.30-34 However, averaged calcium was slightly higher, reflecting the liberal use of calcium containing phosphate binders in this period, and possibly due to development of a dynamic bone disease. Still, this tool can be refined by using variable cut-off levels.

The present study design has some limitations. Retrospective collection of calcium and phosphate data
during the complete period of dialysis is prone to survival bias: only patients alive at the time of IMT/ABI studies were included in the analyses. This is inherent to the present design, but it does not alter the finding of high calcium-phosphate exposure in patients with increased ABI. Secondly, stronger results could possibly have been found if calcium concentrations had been corrected for serum albumin levels. Theoretically, calcium load could have been higher than described, because patients on dialysis can have a low albumin due to their chronic inflammatory state. However, in this patient group albumin concentration was fairly normal, so we assume that correcting for albumin would not have significantly influenced the final results.

For future studies, using calcium concentrations corrected for albumin would be wise. Thirdly, we did not collect the traditional risk factors for arteriosclerosis in these patients longitudinally. However, because of the reverse associations between e.g. weight, hyperlipidaemia and blood pressure and mortality known in ESRD patients and lack of positive intervention studies, one can at least question the role of traditional risk factors in the arteriosclerotic process in ESRD. Finally, of course, data on hyperparathyroidism would have been interesting. During such long dialysis periods many variables such as treatment with vitamin D analogues and parathyroid surgery play a role which are difficult to score objectively.

All in all, we showed that long-term calcium-phosphate exposure in patients with ESRD, assessed by a standardised method, is not associated with IMT and not with decreased ABI, both early markers of arteriosclerosis. We thus found no arguments to state that the vascular changes in ESRD are caused by arteriosclerosis on top of arteriosclerosis. This possibly explains why intervention studies with statins fail to show any benefit in this population.5,6 That calcium-phosphate exposure, measured during long-term dialysis, predicts increased ABI or incompressible ankle arteries gives further support to the opinion that it is mainly arteriosclerosis with media calcification and increased vascular stiffness that is responsible for the increased risk of vascular disease. Using our standardised tool to calculate true calcium-phosphate exposure will help to specify individual risks for patients in predialysis and dialysis periods.

REFERENCES


Cerebral oedema in adult diabetic ketoacidosis: the importance of effective serum osmolality

E.J. Hoorn*, R. Zietse

Dear Editor,

We compliment Haringhuizen and colleagues on their case containing the important message that (fatal) cerebral oedema can also occur during treatment of diabetic ketoacidosis (DKA) in adults.1 We wish to add the following points.

First, we beg to differ that the fall in effective serum osmolality from 391 to 369 mOsm/kg was insufficient to contribute to brain cell swelling. Previously, we have shown that in children an average drop in effective serum osmolality of 9±2 mOsm/kg was associated with cerebral oedema.2 The clinical deterioration at the time of lowest effective serum osmolality also suggests a causal relationship.1

Second, the authors state that ‘progressive hypernatraemia was not completely understood’.1 The course of serum sodium during treatment of DKA is determined by three factors, I) decreasing glycaemia implies the loss of an effective osmole resulting in less water movement from the intracellular to the extracellular compartment resulting in a rise in natraemia, II) the tonicity of the administered intravenous fluids, (III) the ongoing osmotic diuresis, which usually contains more water than sodium. Using a validated formula,3 the fall in serum glucose from 84.9 to 43.2 mmol/l should have led to a rise in serum sodium of ~18 mmol/l, whereas the observed rise was only 10 mmol/l. This implies that the infusate was hypotonic relative to the urine.4 Large infusions are known to induce a natriuresis, a phenomenon referred to as ‘desalination’ and may prevent the necessary rise in serum sodium to prevent a drop in effective serum osmolality.5

These physiological considerations boil down to the following practical points. First, the effective serum osmolality should be used as the primary parameter to guide therapy, as this is the only measure reflecting the opposite trends in glycaemia and natraemia. Second, when the effective serum osmolality falls by ~9 mOsm/kg or more, the risk of cerebral oedema should be anticipated and the infusion rate of insulin and/or saline should be reduced, or, counterintuitively, hypertonic saline should be administered.6 Although the hypertonic state should also be corrected during DKA treatment, the potential complications of cerebral oedema probably outweigh the risks of a hypertonic state in the early phase of treatment. Finally and more generally, this case illustrates that the recommended rates for saline and insulin in the national DKA treatment guidelines are rather high and should be tailored to each individual patient.7

REFERENCES

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The Results should be presented precisely, without discussion.

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