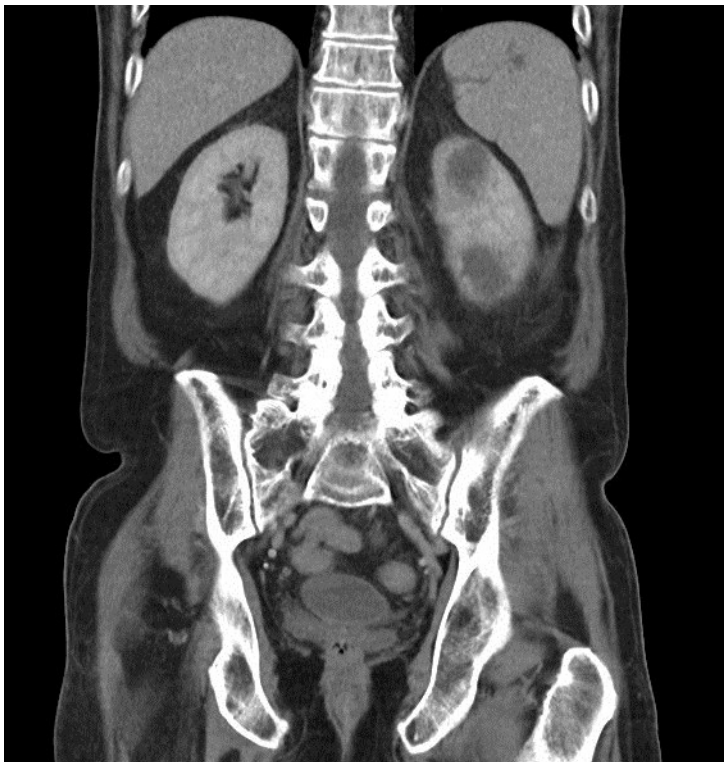
The Netherlands Journal of Medicine

PUBLISHED IN COLLABORATION WITH THE NETHERLANDS ASSOCIATION OF INTERNAL MEDICINE



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may 2015, vol. 73, no. 4, issn 0300-2977

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The Netherlands Journal of Medicine

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ISSN: 0300-2977

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The annual subscription fee within Europe is € 880, for the USA € 921 and for the rest of the world € 1055. Subscriptions are accepted on a prepaid basis only and are entered on a calendar year basis.

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EDITORIAL

Antibiotic use: room for improvement

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Nowadays, appropriate use of antibiotic drugs is not only one of the key issues for infectious diseases physicians in their daily practice but also for policy makers, and the Dutch Minister of Health has put antimicrobial resistance and antibiotic use high on the international agenda. It has long been known that antibiotic therapy and selection of antibiotic resistant micro-organisms are related.[1] The Netherlands and Scandinavian countries are known for their prudent use of antibiotics and low antimicrobial resistance,[2] but also in the Netherlands antibiotic resistant micro-organisms are on the rise.[3] Therefore upon suggestion by the SWAB and endorsed by the Inspectorate of Health, antibiotic stewardship teams (called the A-teams) were installed in all Dutch hospitals in 2014. The question is, however, are we really using antibiotics that badly in the Dutch healthcare system? Total antibiotic use in the Netherlands is one of the lowest in Europe[2] and as shown in the point prevalence study of antibiotic use by Van Spreuwel *et al.*, in this issue of the Netherlands Journal of Medicine, more than 75% of all antibiotics are prescribed according to the guidelines.[4] Although we seem to be doing well, results of this point prevalence study still identify areas for improvement. Since the study was done in a tertiary hospital, it is not clear whether the results can be extrapolated to other hospitals in the Netherlands.

First, the 75.7% adherence to the guidelines seems high in comparison with other studies that measured antibiotic use. A point prevalence study in an Australian tertiary hospital showed 47% inappropriate use of antibiotic drugs, and in a study in a large Dutch teaching hospital study 37% inappropriate drug use was reported.[5,6] The high adherence to the guidelines in Nijmegen may be explained by differences in country and the time the study was performed. Another explanation may be that for 33% of patients an infectious diseases specialist was involved in the prescription of antibiotics and in at least 15% of the prescriptions the advice of the infectious diseases specialist was considered to be similar to adherence to the guidelines.[4] Involvement of infectious diseases specialists has been shown to increase adherence to guidelines.[4,7] Therefore, the adherence percentage in Nijmegen may be higher than in other hospitals in the Netherlands, especially those hospitals without an infectious disease (ID) consultation service.

Antibiotic drug use in the Netherlands is high, in the PREZIES network it has been shown that 32% of all admitted patients receive antibiotic medication[8] and in Nijmegen even 41% of the admitted patients used antibiotic drugs. Improving prescription of these often used medications is not only necessary to halt the increasing antimicrobial resistance, but also to reduce drug toxicity and costs. Areas of antimicrobial stewardship should also include dosing of antibiotics, especially in the presence of renal insufficiency or co-medication, duration of (intravenous) therapy and switch from intravenous to oral antibiotic therapy; these areas were not addressed.[4] In Nijmegen, use of amoxicillin-clavulanic acid and having a respiratory tract infection were associated with less adherence to the guidelines. Other studies showed that bone/joint infections, creatinine level > 120 mmol/l, carbapenem, macrolide and fluoroquinolone use and being under the care of the rehabilitation team were risk factors.[5,6] These last two studies used the method developed by Gyssens *et al.*[5,9] to define inappropriate use of antibiotic therapy. The difference in results in identification of areas of improvement suggests that hospitals should at least confirm that the areas for improvement of antibiotic therapy reported above are problematic in their wards too.

Another area of concern is empirical therapy, since only 42 of the 230 prescribed antibiotic drugs (18%) were directed at a known pathogen.[4] Empirical therapy may be too broad, resulting in more antibiotic resistance. On the other hand, up to 47% of empirical antibiotic therapy in the Netherlands was deemed to be inappropriate without ID consultation, decreasing to 25% when an infectious diseases specialist was in consultation before the start of therapy.[9] Start of empirical therapy may be a difficult area to address without overloading infectious diseases specialists, but computer-assisted clinical decision support systems may be used for this purpose in the future.[11]

Taken together, identifying areas for improvement of antibiotic use is important for antibiotic stewardship with the aim of reduction of the percentage of inappropriate empirical therapy, adverse drug events, costs and increasing narrowing down of antibiotic drugs. As shown by Van Spreuwel *et al*. point prevalence studies of antimicrobial use are feasible and give valuable information for the A-teams and may be a tool to measure their effectiveness. As with surveillance of antimicrobial resistance, evaluation of antibiotic use is a prerequisite for antibiotic stewardship.

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REVIEW

Non-cystic fibrosis bronchiectasis: clinical presentation, diagnosis and treatment, illustrated by data from a Dutch Teaching Hospital

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ABSTRACT

This review article describes the epidemiology, clinical presentation, diagnostic workup and treatment options in adult non-cystic fibrosis (non-CF) bronchiectasis (widening of mainly small and medium-sized bronchi as seen on chest computed tomography (CT) scan). We illustrate evidence from the literature with our own data retrieved from chart review, involving 236 adult patients with recurrent lower respiratory tract infections and high-resolution CT-proven non-CF bronchiectasis, who visited the outpatient clinic for respiratory diseases of a large Dutch teaching hospital between 2000 and 2010.

Non-CF bronchiectasis can be described as a final common pathway of a vicious cycle of excessive bronchial inflammation, bacterial colonisation and infection. Non-CF bronchiectasis may arise from several causes, headed by infection and immunodeficiency, and is clinically characterised by a chronic, productive cough and infectious exacerbations. Once non-CF bronchiectasis is diagnosed using high-resolution CT scanning, a protocol-driven work-up to identify the underlying cause is recommended. Non-medicinal treatment options are primarily directed at clearance of bronchial secretions, which can further be improved by inhalation of hyperosmolar agents. Antibiotic treatment of exacerbations is a cornerstone medicinal treatment in bronchiectasis management. Patients with frequent exacerbations can be considered for long-term low-dose macrolide treatment, supported by robust evidence. Inhaled antibiotics might be beneficial in selected patients colonised with *Pseudomonas aeruginosa*. Important developments in the last decade include the introduction of international guidelines and the proposal for a validated scoring system for disease severity. Bronchiectasis patients are encountered by physicians in diverse medical professions and the disease itself is still underdiagnosed. The authors aim to increase awareness of the condition and provide practical tools for diagnosis and treatment.

KEYWORDS

Diagnostic workup, epidemiology, maintenance treatment, non-cystic fibrosis bronchiectasis treatment

INTRODUCTION

Bronchiectasis – characterised by irreversible, pathological dilatation of the small and medium-sized bronchi – is not a disease in its own right, but rather a final common pathway of a vicious cycle of inflammation, bacterial colonisation and infection. A variety of respiratory and systemic diseases may be complicated by pathological bronchial dilatation, and therefore various medical specialists will be dealing with the condition in one way or another. Although general availability of computed tomography (CT) scans has importantly contributed to higher case-finding rates, bronchiectasis is still considered an underdiagnosed condition. In this article, we address the different signs and symptoms which can be clues to the diagnosis in order to facilitate recognition of the disease among non-pulmonary physicians. We further discuss our preferred diagnostic approach and give an overview of evidence-based treatment options.

Cystic fibrosis, an inherited multi-system disorder, is usually discussed separately and here we focus on non-cystic fibrosis bronchiectasis – hereafter referred to as 'bronchiectasis'. The gold standard for diagnosis has long been bronchography, until the introduction

of high-resolution CT scanning, the current standard diagnostic test. Due to the abundant amount of purulent phlegm produced by affected individuals, bronchiectasis was considered offensive and also untreatable before the introduction of antimicrobial agents.[1]

Around World War I, bronchiectasis was common in the Western world and it carried a poor prognosis: over 40% of all patients died of respiratory causes before the age of 40.[2-4] Improved socio-economic status, successful nationwide vaccination programs for whooping cough and measles, and – most importantly – the availability of antibiotics reduced both incidence and mortality, in developed countries at least. Indeed, bronchiectasis became an 'orphan disease', as a result of which the focus of clinicians and researchers diverted away from this condition, which was now considered rare with a relatively benign course.

In spite of adequate antibiotic treatment, however, bronchiectasis still has the potential to cause substantial morbidity, including repeated lower respiratory infections complicated by haemoptysis, a disabling productive cough and shortness of breath, all of which importantly affect quality of life.[2] Patients with bronchiectasis were found to spend more days in hospital and have higher annual medical care expenditure as compared with matched controls.[3]

Recent epidemiological studies show a high incidence of bronchiectasis among New Zealand's and Australia's indigenous population and inhabitants of remote areas in Alaska.[4] In the developed world estimated prevalence ranges from 0.42 per 100,000 in 18-34 year olds to 272 per 10,000 in those over 75.[5]

Important developments in the last decade include the introduction of international guidelines, the proposal for a validated scoring system for disease severity and the first large randomised trials on antibiotic maintenance treatment for those with frequent exacerbations, all of which will be discussed in this article.[6-9]

We illustrate the evidence from the literature on the diagnosis and treatment of bronchiectasis using the experience gained in a large Dutch teaching hospital. Demographic, epidemiological and clinical data were collected from the entire, unselected, non-CF bronchiectasis cohort of the Alkmaar Medical Centre in 2010, for research purposes. Data were retrieved from chart review of all adult patients with recurrent lower respiratory tract infections and high-resolution CT-proven non-CF bronchiectasis who visited the outpatient clinic for respiratory diseases of the Medical Centre Alkmaar at the time.

PATHOPHYSIOLOGY

The mechanism of disease that eventually causes bronchiectasis is traditionally depicted as a vicious circle of excessive inflammation and bacterial colonisation. Diverse stimuli, which can be either endogenic (such as ciliary defects) or exogenic (e.g. foreign body aspiration), may result in structural damage to the airways. This in turn allows for persistent bacterial colonisation of the larger and medium-sized bronchi. The host inflammatory responses together with secreted bacterial toxins cause additional damage (hypersecretion, ciliary dysfunction and airway remodelling) which further weakens local resistance.[10,11]

The immune response in bronchiectasis is mainly neutrophil driven and increased levels of chemokines and pro-inflammatory cytokines are found in the airways of affected individuals.[12,13] High levels of proteases – toxic neutrophil products excreted on neutrophil activation – are present at the site of inflammation, resulting in release of pro-inflammatory cytokines and exerting proteolytic activity, thus causing even more damage to cells constituting the structure of the airways.[14] T-cell infiltration, impaired macrophage phagocytosis, altered epithelial cell function and, more recently, deficiency of mannose-binding lectin have all been proposed as additional mechanisms responsible for an enhanced inflammatory response.[11,15-18] A cycle of oxidative stress is also present, in which (mainly neutrophil derived) reactive oxygen species cause damage to cells and the surrounding tissues and induce additional oxidative stress through activation of the inflammatory transcription factors nuclear factor-kappa B and activator protein-1.[19]

CAUSES

Bronchiectasis may arise from several different causes, headed by infection and immunodeficiency, mostly primary antibody deficiency syndromes (*table 1*). Due to successful prevention programs for tuberculosis and childhood infections such as whooping cough and measles, post-infectious bronchiectasis tends to become less common in developed countries. In about half of the patients, no underlying cause of permanent airway damage is found. Shoemark et al.[20] found no causative factor in one third of their patients despite thorough systematic investigations in a tertiary referral centre. Other centres with multidisciplinary specialised bronchiectasis outpatient clinics with diagnostic protocols in place report 40-50% idiopathic bronchiectasis in spite of an extensive workup.[21-24]

Bronchiectasis is seen in 7-25% of patients with asthma or chronic obstructive pulmonary disease (COPD), coinciding with more severe disease.[25,26] While asthma has recently been considered a cause of bronchiectasis in the absence of other factors, the link between COPD and bronchiectasis has yet to be established.[6]

The underlying cause for our cohort of patients is shown in *table 1*.

Table 1. *Aetiology of bronchiectasis in 236 patients visiting the outpatient department of the Alkmaar Medical Centre as compared with possible causes for bronchiectasis as found in non-CF bronchiectasis phenotyping studies and clinical trials, n (total) = 1535*[20-22,24,55,60]

	Literature (n = 1535)	Alkmaar cohort (n = 236)
Post infectious • Non-tuberculous mycobacteria • Tuberculosis • Pneumonia • Childhood infections (e.g. pertussis, measles, adenovirus)	20- 38%	17.4%
Immunodeficiency *Primary* • Hypogammaglobulinaemia • X-linked agammaglobulinaemia *Secondary* • Leukaemia • HIV / AIDS • Following chemotherapy or immunosuppressive therapy	3% - 24%	7.1%
Asthma	3-11%	11.4%
Allergic bronchopulmonary aspergillosis	3-8%	3.0%
Mechanical obstruction • Tumour • Foreign body • Lymphadenopathy	0-1%	0.4%
Sequelae of inhalation or aspiration • Gastro-oesophageal reflux • Inhalation of toxic fumes	1-4%	2.5%
Auto-inflammatory conditions • Rheumatoid arthritis • Sjögren's syndrome • Systemic lupus erythematosus • Ulcerative colitis or Crohn's disease	2-3%	4.7%
Congenital conditions • Cystic fibrosis • α1 anti-trypsin deficiency • Primary ciliary dyskinesia • Kartagener syndrome (situs inversus, chronic sinusitis, bronchiectasis) • Mounier-Kuhn syndrome (tracheobronchomalacia) • Williams-Campbell syndrome (cartilage deficiency)	1-18%	4.2%
Other uncommon aetiologies • Yellow nail syndrome (yellow nails and lymphedema) • Young's syndrome (sinusitis-infertility syndrome) • Diffuse panbronchiolitis	1-3%	0.4%
Idiopathic	26-56%	47.9%

CLINICAL PRESENTATION AND SYMPTOMS

The 'typical' patient with bronchiectasis is supposedly a middle-aged woman, who is a lifelong non-smoker – or at least, this is the profile of the majority of patients in bronchiectasis phenotyping studies.[20-23,27,28] Our own data do not completely reflect this picture, as our patients were slightly older and more frequently smokers (*table 2*). This incongruence illustrates the varied clinical presentation of bronchiectasis patients in clinical practice. Bronchiectasis can just as well occur in the 80-year-old male with frequent and virulent exacerbations of obstructive lung disease as in the 40-year-old lady with rheumatoid arthritis visiting your practice with complaints of persisting cough. Severity of symptoms is different for each patient, but in general the course of the disease is highly variable, including nearly symptom-free periods interspersed with infectious exacerbations. The most persistent and often presenting symptom is a chronic productive cough, present in 96% of 103 patients referred to a pulmonary outpatient clinic, with the amount of sputum being among the main determinants of quality of life.[2] Dyspnoea, fatigue and upper respiratory tract symptoms are encountered in 60-70% of patients. About half of the patients describe having specks of blood in their sputum at any time, but haemoptysis resulting in immediate medical consultation is present in a quarter of patients. Pleuritic or musculoskeletal chest pain is present in 25-50% of patients and chest pain is often the reason for repetitive investigations at emergency departments. Exacerbations are characterised by an increase in symptoms and signs suggesting lower respiratory tract infection. Physical examination is often unremarkable except for the presence of crackles, mostly bilateral at the lower lobes.[27,28]

DIAGNOSTIC WORKUP

Bronchiectasis ought to be considered in patients with a chronic productive cough and/or recurrent lower airway infections, especially when these symptoms are present in younger, non-smoking individuals. Haemoptysis, recurrent para-nasal sinus infections or successive sputum cultures positive for *S. aureus* or *P. aeruginosa* may also be clues leading to the diagnosis. In patients with asthma or COPD, bronchiectasis should be considered in case of frequent, slow-resolving exacerbations, unstable or medication-resistant asthma or severe symptoms despite limited exposure to smoking in patients diagnosed with COPD.[6]
Key to the diagnosis are imaging studies using high-resolution CT. The chest CT protocol should be a spiral CT with 1 mm slices, able to detect pathology of larger and smaller airways, preferably with software

Table 2. *Patient characteristics (n = 236) from patients with recurrent lower respiratory tract infections and non-CF bronchiectasis in a large Dutch teaching hospital*

Female sex – No. (%)	154 (65.3)
Age – year	65.7 (57.4-75.1)
Never smoker – No. (%)	134 (56.8)
Current smoker – No. (%)	15 (6.4)
FEV-1 - % of predicted	87 (66.0-103.0)
FVC - % of predicted	97 (79.0-110.0)
Age at first presentation – year	58.3 (47.0-65.5)

Continuous variables are presented as median (IQR). FEV-1 = forced expiratory volume in the first second; FVC = forced vital capacity.

allowing reconstruction in different planes. In patients with bronchiectasis, high-resolution CT typically shows a distorted ratio (> 1.0) of the inner bronchial diameter as compared with the accompanying artery, and signs of bronchial dilatation: lack of tapering and increased visibility of small airways in the sub-pleural region (*figure 1*).[29] Plain chest X-rays show abnormalities in a large proportion of bronchiectasis patients (66% of our cohort), but changes are non-specific and an unremarkable chest X-ray does not rule out bronchiectasis.

In symptomatic patients, the radiological finding of bronchiectasis should be followed by investigations to reveal the underlying cause. If a standardised protocol is used, the diagnostic yield may be enhanced, resulting not only in reduction of the proportion of patients diagnosed with 'idiopathic' bronchiectasis, but even in changing the treatment and the prognosis in up to 50% of patients.[30,31] We use a diagnostic algorithm based on national and international guidelines (*figure 2*).[6,32,33]

A standardised workup has been shown to reduce diagnostic delay, which could last for up to several years, especially in patients with underlying immune deficiency.[34] Localised bronchiectasis is usually indicative of a local mechanical cause (e.g. middle lobe syndrome) or post-infectious damage. The latter is even more plausible when a clear temporal relationship exists between an infectious episode and development of bronchiectasis-related symptoms. In other subjects, bronchiectasis can occur as a symptom of an already identified disease, such as rheumatoid arthritis or inflammatory bowel disease. In such cases we suggest to refrain from extensive investigations – or to only resort to additional testing if unexplained deterioration occurs. The same holds true for patients with asymptomatic bronchiectasis, as for instance can be seen in stable fibrosis (traction bronchiectasis).

TREATMENT OPTIONS

When a specific disorder is found to cause bronchiectasis, disease management should primarily be directed at the underlying cause. This, for instance, applies to bronchiectasis due to allergic bronchopulmonary aspergillosis or common variable immune deficiency, both requiring their own treatment regimens.

Bronchiectasis management is aimed at preventing disease progression and improving quality of life by reducing symptoms and exacerbations. This includes treatment of exacerbations and optimal airway clearance, complemented with long-term antibiotic therapy (oral or nebulised) or surgery in selected cases. Many treatment options for non-CF bronchiectasis are derived from the treatment regimens developed for cystic fibrosis. At first, treatment modalities were simply extrapolated to non-cystic fibrosis patients, but in the last decade, treatment modalities have been studied for this specific group of patients, resulting in evidence-guided treatment recommendations. Sometimes

Figure 1. *CT scans of bronchiectasis; three patients showing typical radiological features of bronchiectasis: A. Increased bronchial diameter (signet ring sign) in a patient with allergic bronchopulmonary aspergillosis. B. Lack of tapering in a patient with COPD complicated by bronchiectasis. C. Increased visibility of small airways in the subpleural region in a patient with rheumatoid arthritis-associated bronchiectasis*

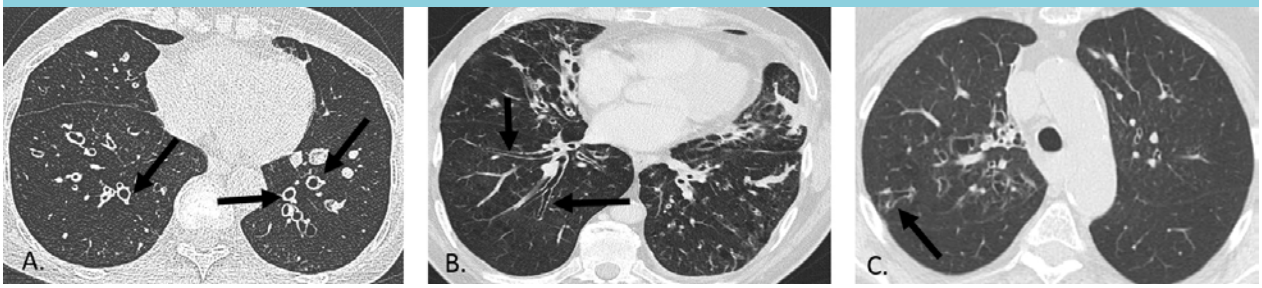
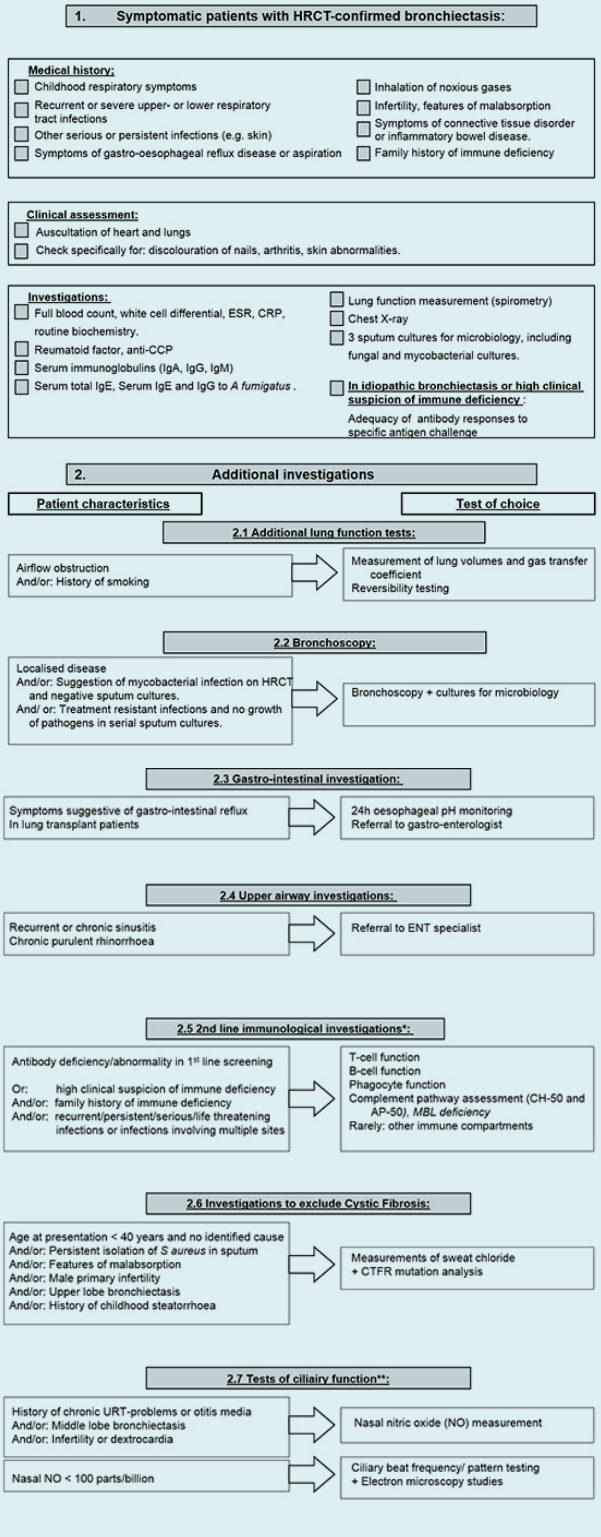


Figure 2. *Diagnostic workup in adult bronchiectasis patients*



*always in conjunction with clinical immunologist
**only to be performed in specialist centre

these recommendations contradict those for cystic fibrosis, as is true for mucolytic treatment with recombinant human DNAse (rhDNAse). Routinely used in cystic fibrosis treatment, rhDNAse was found of no benefit in one trial of non-CF bronchiectasis and harmful in another.[35] Insufficient evidence is available to support the use of other mucolytics, such as acetylcysteine, in non-cystic fibrosis patients. Inhaled corticosteroids – although widely used by non-specialists in non-CF bronchiectasis patients – were only found effective in patients with underlying asthma. Current guidelines advise against routine use in non-CF bronchiectasis.[6]

It is worth mentioning that the pharmacological options described below – such as macrolides or inhaled hyperosmolar agents – have been approved by neither the US Food and Drug Authorization nor the European Drug Regulators. Use is solely based on outcomes of clinical trials and international guidelines.

Management of infectious exacerbations

One of the cornerstones of bronchiectasis management is antibiotic treatment of infectious exacerbations. There are no randomised trials evaluating the effect or the duration of antibiotic treatment in bronchiectasis, but antibiotics are generally thought to reduce the time to recovery and to reduce symptoms. By convention, a 14-day course of antimicrobials is prescribed, either intravenously or orally for exacerbations that last several days at least and are accompanied by increased sputum purulence, volume or reduced viscosity and increased cough, dyspnoea and systemic upset such as fatigue or fever.[6]

Preceding antibiotic treatment, sputum samples should be submitted for microbiological investigation and therapy should be directed at previously or newly isolated pathogens.

Physiotherapy

Most patients with bronchiectasis, especially those with excessive secretions, are offered physiotherapy. A customary physiotherapy program in the Netherlands would include one or more techniques directed at improved clearance of broncho-pulmonary secretions, combined with a pulmonary rehabilitation program to improve exercise tolerance. Forced expiratory manoeuvres as well as hand-held devices generating positive expiratory pressure ('pep' devices) such as Flutter™ or Acapella™ are used for optimal sputum clearance. A recent randomised trial, evaluating a similar approach, demonstrated a beneficial effect on exercise capacity, dyspnoea and fatigue in 85 patients.[36]

A Cochrane review, evaluating the effect of physiotherapy-taught airway clearance techniques (ACT) as compared with no therapy or active coughing, demonstrated small

improvements in sputum expectoration, lung function and health-related quality of life in five small and diverse studies, involving 51 patients.[37] The choice of an ACT might as well be guided by patient preference, since there is no clear evidence in favour of any of the ACTs available. A small randomised study in 30 patients showed improved exercise tolerance and health-related quality of life with pulmonary rehabilitation in addition to ACT as compared with ACT alone.[38]

Inhalation of hyperosmolar agents

Due to impaired mucociliary clearance, many patients with bronchiectasis suffer from mucus hypersecretion and retention, leading to dyspnoea, chronic cough and increased susceptibility to infections. We frequently use inhalation of isotonic (0.9%) or hypertonic saline (6-7%) twice daily in addition to airway clearance techniques for optimal sputum evacuation. An evident benefit of nebulised hypertonic saline over isotonic saline has not yet been demonstrated in the small studies available and in our experience, patients report less discomfort in terms of wheezing or dyspnoea when using the isotonic solution.[39] Nevertheless, the inhalation process itself is often experienced as time consuming and inconvenient.

The hyperosmolar agent mannitol reduces exacerbations and improves lung function in cystic fibrosis.[40] When administered as dry powder through a purpose-designed inhaler device, it is proposed as a less cumbersome alternative to saline inhalation. Several smaller or short-term studies on mannitol inhalations in bronchiectasis yield conflicting results in terms of sputum expectoration and quality of life.[39] The sole large – yet slightly underpowered – long-term trial of 400 mg mannitol twice daily vs. a non-therapeutic dose of 50 mg demonstrated that inhaled mannitol increases the time until first exacerbation in patients with bronchiectasis, without improving respiratory quality of life or reducing actual exacerbation rates.[41] Mannitol is known for inducing bronchospasm. It is worth noting that all participants in two large clinical trials were screened for mannitol tolerance at baseline and excluded when mannitol-induced bronchospasm was present (in 16% of all screened subjects). In the other participants mannitol inhalations were safe and well-tolerated.[41,42] In the Netherlands, dry powder mannitol (Bronchitol™) is primarily used for optimising sputum expectoration in cystic fibrosis patients and is not registered for use in other patient groups.

Long-term antibiotic treatment

Treatment with maintenance antibiotics in bronchiectasis can be directed at simply reducing the increased bacterial load, since chronic colonisation has been found to coincide with enhanced inflammation and worse clinical outcome. In case of macrolides it is thought to dampen the exaggerated inflammatory response through multiple pathways.[43]

Macrolides

Macrolides, because of their anti-bacterial and anti-inflammatory properties, have long been thought ideal to intervene in the vicious circle of infection and inflammation that underlies bronchiectasis. In three different clinical trials evaluating long-term oral macrolide treatment, exacerbation frequency was significantly reduced. All trials used different dosing regimens and there is an ongoing debate on which schedule should be used. Traditionally, many physicians use a dosing schedule equivalent to the cystic fibrosis treatment schedules consisting of azithromycin 500 mg thrice weekly or 250 mg daily. Similar schedules were used in the BAT and EMBRACE trials, as opposed to the Australian BLESS trial which used erythromycin 400 mg twice daily.[7-9] In cystic fibrosis patients macrolide antibiotics, and in particular azithromycin, tend to cumulate inside alveolar macrophages and as such have an extended half-life. Based on the pharmacokinetic properties of azithromycin in cystic fibrosis patients – whose kinetics may differ considerably from those without cystic fibrosis – dose levels of 22-30 mg/kg/week divided by 1-7 dosing moments, are proposed.[44] Lung function improvement and enhanced quality of life were most distinct in patients with frequent exacerbations. Recent COPD trials show a tendency to a higher yield of macrolide treatment in patients with more exacerbations.[45] Although bronchiectasis guidelines consider patients with three or more exacerbations yearly and suffering from chronic symptoms to be candidates for this treatment type, no robust evidence is as yet available to justify abstaining from macrolide treatment in less frequent exacerbators.[7-9]

Benefits of macrolide treatment come with a considerable increase in macrolide-resistant pathogens, which demands judicious use of long-term macrolide therapy.

Inhaled antibiotics

Since the late 1990s, nebulised antibiotics for reducing airway bacterial load have been considered a treatment option in bronchiectasis. Higher bacterial load is found to coincide with augmented systemic inflammation and increased morbidity.[46] Due to the favourable pharmacokinetic profile of inhaled substances, with minimal systemic drug delivery, systemic adverse effects are mild.[47,48] Local, non-severe side effects are frequently encountered in clinical trials with inhaled antibiotics.[49] Inhalation-induced bronchospasm could pose an extra challenge in clinical practice, but is usually overcome through inhalation of a short-acting beta-2 agonist prior

to inhalation of antibiotics. Most randomised clinical trials evaluating inhaled antimicrobial agents included bronchiectasis patients colonised with *Pseudomonas aeruginosa* and used different types of antibiotics (colomycin, tobramycin, amikacin, or ciprofloxacin).[50-54] In addition, the three distinct trials (using aztreonam, gentimicin and ciprofloxacin), which did not specifically require *P. aeruginosa* colonisation for inclusion, in fact included many patients with *P. aeruginosa* colonisation at baseline (48-85%).[49,55,56] All trials demonstrated bacterial load reduction in the airways of actively treated patients, but this effect does not correspond consistently with improvement in clinical endpoints.[57] The largest trial (n = 500) of inhaled aztreonam in bronchiectasis patients – 85% of whom were *P. aeruginosa* colonised – failed to demonstrate reduced exacerbation rates or improved quality of life.[49] Other authors report prolonged time to exacerbation and improved health-related quality of life as secondary findings. The attractive safety profile and encouraging results in some studies have stimulated further research in this field and momentarily no less than seven trials are recruiting patients, most of which studying inhaled ciprofloxacin.[58]

Awaiting further evidence we think that nebulised antibiotics offer a reasonable alternative to oral treatment in selected patients colonised with *P. aeruginosa*.

Other non-pharmacological options, such as surgery for localised disease and bronchial artery embolisation in case of massive haemoptysis, will not be discussed here in detail.

PROGNOSIS

Although bronchiectasis can cause considerable morbidity, prognosis in terms of survival is favourable. The largest prospective study up until now found 62 deaths in 608 patients (10.2%) within four years, but the majority of deaths (81%) occurred above the age of 70.[59] Independent predictors of mortality were older age, low FEV-1, prior hospitalisation and three or more exacerbations in the year prior to the study. The authors used these data to compile and validate a clinical prediction tool, the Bronchiectasis Severity Index, which divides patients into three risk groups (low/ intermediate/ high) in order to predict mortality, hospital admissions and exacerbations.

This tool could be very useful in research settings in order to increase homogeneity of study populations. Its value for directing therapy in a clinical setting still needs to be proven.

In conclusion, the broad range of diseases that cause or coincide with bronchiectasis make it a frequently encountered entity in various medical specialisations. The authors hope that this article will renew awareness of this still underdiagnosed condition. Exciting new developments are the publication of high-quality, randomised studies and new tools for patient selection which are important steps towards improving bronchiectasis management.

DISCLOSURES

The authors declare no conflicts of interest. No funding or financial support was received.

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REVIEW

Seoul hantavirus in brown rats in the Netherlands: implications for physicians

Epidemiology, clinical aspects, treatment and diagnostics

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ABSTRACT

The recent discovery of Seoul hantavirus (SEOV) presence in wild rat populations in the Netherlands has direct implications for Dutch clinicians and hantavirus diagnostics. SEOV is amongst the Old World hantaviruses which cause haemorrhagic fever and renal syndrome (HFRS) in humans. HFRS is characterised by a classical triad of fever, acute kidney injury and haemorrhage, but can show different signs and symptoms in specific cases. SEOV is transmitted from infected rats to humans by inhalation of aerosolised excreta. When compared with the known circulating hantaviruses in the Netherlands, Puumala (PUUV) and Tula (TULV), SEOV causes a more severe form of HFRS. Data from cohort studies undertaken in China and Northern Europe show differences in signs and symptoms at onset of disease, (haemorrhagic) complications and mortality. Furthermore, routine diagnostics currently available for hantavirus diagnosis in the Netherlands are not optimised for SEOV detection. The clinical outcome of an SEOV and PUUV infection will greatly benefit from an early diagnosis which will reduce the costs of unnecessary tests and treatments as well. The discovery of SEOV circulation in the Netherlands follows recent findings of SEOV infections in both rodents and humans in England, Wales, France, Belgium and Sweden, indicating the emerging character of SEOV and a high importance of this hantavirus for Public Health in large areas of Europe. Here, we review the current knowledge on the clinical manifestation of SEOV versus PUUV infections in humans, the treatment of clinical cases and diagnostics.

KEYWORDS

Haemorrhagic fever with renal syndrome, Seoul virus, viral haemorrhagic fever, zoonoses

BACKGROUND

In January 2015, the first conclusive evidence was presented for the circulation of Seoul hantavirus (SEOV) in wild brown rats (*Rattus novergicus*; Norway rats) in the Netherlands.[1] The report of circulation of SEOV in the Netherlands followed recent findings of SEOV infections in humans and rats in other North-Western European countries and underlines the emerging character of SEOV-related disease in Europe. SEOV can cause haemorrhagic fever with renal syndrome (HFRS) in humans and is transmitted from rats to humans by inhalation of aerosolised excreta from infected rats.[2-4] Until now, there has only been evidence for the circulation of two other hantaviruses in wild rodents in the Netherlands, namely Puumala virus (PUUV) in bank voles (*Myodes glareolus*) and Tula virus (TULV) in common voles (*Microtus arvalis*),[5] with an annual incidence of notified clinical human PUUV cases varying between 4 and 24.[6] Evidence for underdiagnosis of infections with hantaviruses in the Netherlands exists with a lack of awareness for HFRS among physicians as a likely explanation.[6,7] The presence of SEOV in rat populations in the Netherlands has direct implications for the clinician and routine hantavirus diagnostics. Here, we review the current knowledge on the clinical manifestation of SEOV

versus PUUV infections in humans, the treatment of clinical cases and diagnostics.

EPIDEMIOLOGY

Hantaviruses (family *Bunyaviridae*, genus *Hantavirus*) are the aetiological agents of HFRS in Eurasia, and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. HFRS is diagnosed in more than 10,000 individuals in Europe annually and the recorded numbers of hantavirus infections in Europe have been steadily increasing during the last 20 years.[3,4] Hantaviruses are carried by rodents, insectivores (Soricomorpha) and bats, and most hantaviruses are restricted to a single reservoir host species. Hantaviruses pathogenic to humans are all associated with rodent reservoirs and humans become infected through inhalation of aerosolised excreta from infected rodents.[4] The majority of HFRS cases in Europe are caused by PUUV hosted by the bank vole (*Myodes glareolus*).[4] In addition four genotypes of the Dobrava-Belgrade virus (DOBV) can be found across Europe, each associated with its specific *Apodemus* spp. and causing HFRS with different degrees of severity. These include DOBV-Aa carried by *A. agrarius*, which is recognised by the International Committee on Taxonomy of Viruses as a unique hantavirus species, Saarema virus (SAAV). The classification of *Apodemus*-borne hantaviruses is still under debate, hence SAAV and DOBV-Aa are both used in the literature to indicate *A. agrarius* associated hantavirus.[8,9]

The specific relationship between hantaviruses and their carriers makes host ecology the deciding factor in the geographic distribution of these viruses, resulting in a clear distinction between Old World and New World hantaviruses.[10] SEOV is the only hantavirus with a worldwide distribution as its main reservoir, the brown rat is omnipresent due to global trade and human migration in the past centuries.[11,12] In Europe, evidence for SEOV circulation in brown rats has been accumulating in the past two years with molecular evidence in wild rats in France, Belgium and the United Kingdom (UK), and in pet rats in the UK and Sweden.[13-19] Besides a few reports on zoonotic transmission of SEOV through handling of laboratory rats in the 1980s and 1990s in Belgium, France, the Netherlands and the UK,[17] reports on human infections with SEOV outside Asia are rare and limited to urban settings.[20-22] The first non-laboratory related infections with SEOV that were reported in Europe were in a farmer in the UK and a pregnant woman in France in 2012, both in rural settings and most likely due to indirect contact with wild brown rats.[15,23,24] A seroprevalence study among farmers in the same region in the UK suggested a widespread rural circulation of SEOV.[25] In 2013, three human SEOV cases were reported among pet rat owners in the UK.[13,26] In 2014 serological and molecular evidence was found for the circulation of SEOV in wild brown rats in a region in the east of the Netherlands.[1] However, an investigation in a syndromic cohort in the Netherlands in 2010 and 2011 did not yield evidence for human SEOV infections in the Netherlands.[7]

VIROLOGY

Hantaviruses are negative-stranded, enveloped viruses with a tri-segmented RNA genome. The large segment (L) codes for the RNA polymerase, the medium segment (M) codes for the surface glycoproteins Gn and Gc, and the small segment (S) codes for the nucleocapsid protein. The termini of these RNA segments contain conserved regions and are often used as targets for detection of hantaviruses in patients and reservoir hosts.[4]

CLINICAL MANIFESTATION

Haemorrhagic fever with renal syndrome

The classic presenting symptoms, often referred to as the HFRS triad, of Old World hantavirus infection, are the combination of acute kidney injury (AKI) and fever which could both potentially be accompanied by (severe) bleeding complications.[2] Although bleeding complications only occur in a minority of HFRS patients (namely 5-60% of the symptomatic cases depending on the causative hantavirus species) HFRS-causing hantaviruses are generally classified among the viral haemorrhagic fever pathogens.[27,28] In general, five phases can be recognised in the disease course of HFRS: first, after an incubation period varying between two to three weeks, patients enter a febrile phase characterised by high fever (> 39 °C) accompanied by aspecific 'flu-like' symptoms such as myalgia and a severe headache. This phase is followed by a hypotensive state which is very likely to be the result of inadequate vascular tone and increased vascular permeability associated with pathological findings of pulmonary and retroperitoneal oedema.[27] After this shock-like condition patients could develop oliguria which is followed by a diuretic phase and eventually convalescence.[2,27] Of the three hantaviruses known to circulate in the Netherlands, PUUV causes a mild HFRS, which also seems to be the case for TULV, although evidence for the clinical course of TULV in human remains scarce. Based on data from Asia and case reports in Europe, SEOV seems to cause a moderate HFRS with, in general, a more severe clinical outcome of the disease when compared with PUUV and TULV.

Puumala virus: mild HFRS, nephropathia epidemica

Until now the basic assumption in the Netherlands is that all cases of HFRS are caused by PUUV, although infection with TULV cannot be excluded due to the use of serology-based diagnostics (see below). The clinical course and outcome of PUUV have been relatively well studied in Europe.[29,30] The majority of PUUV infections are asymptomatic (70-80%) and PUUV HFRS is considered a 'mild' form of HFRS, often referred to as *nephropathia epidemica*.[31] Most of the symptomatic patients present with AKI, fever and limb and back pain potentially accompanied with nausea and vomiting. The case fatality rate (CFR) for PUUV varies at around 0.1% and is especially associated with the age of the infected individual. Most nephropathia epidemica deaths occur in older persons and fatalities in patients below 50 years of age are rare.[32] The percentage of clinical cases that develop haemorrhagic complications is estimated to lie between 1-5%. Both CFR and the percentage of clinical cases with bleeding complications are considerably lower when compared with other Old World hantaviruses, such as SEOV, which cause moderately severe HFRS (see below).[29,31] Laboratory analysis most often reveals a clear thrombocytopenia combined with increased creatinine levels. Furthermore, the leukocyte count is elevated and left-shifted combined with elevated C-reactive protein levels. Sporadic long-term complications of nephropathia epidemica include hypertension, proteinuria and persistent haematuria.[29] PUUV infections have been notifiable in the Netherlands since December 2008.

Tula virus: unclear, most likely mild HFRS

Descriptions of the clinical course of TULV infection in humans are rare. The detection of TULV-specific antibodies in German forestry workers and healthy blood donors in the Czech Republic strongly suggests that TULV can be transmitted to humans. Furthermore, TULV infection resulted in an HFRS-like syndrome with severe lung involvement in an immune compromised patient in the Czech Republic. Another TULV case report describes a period of fever and exanthema in a patient bitten by a rodent.[33-36]

SEOV: moderate HFRS

The specific course of HFRS caused by SEOV is less well studied than that of PUUV and some discrepancies between studies are present. As in nephropathia epidemica, most patients present with the classic triad of fever, renal insufficiency and possibly accompanied by bleeding symptoms. Of importance is the high number of patients that also show gastrointestinal symptoms at the time of presentation. SEOV is classified as causing a 'moderate' form of HFRS with a CFR of 1-2% when compared with severe HFRS caused by DOBV and Hantaan virus (HTNV), which have a CFR > 10%.[37] Multiple cohort studies (mainly from China) describe signs of haemorrhage (petechia, haematuria and epistaxis) in about 50% of the patients diagnosed with SEOV-caused HFRS, which is remarkably higher than the 5% reported for PUUV, but lower than the 70-80% reported for HNTV and DOBV.[38,39] A recent case report of a SEOV infection in France described severe disease with signs of haemorrhage and increased liver enzymes in a pregnant woman.[24] Increased liver enzyme levels are of interest since these were also present in the other European SEOV case in the United Kingdom.[23] The pronounced elevation of the liver enzymes made the treating physicians first suspect viral hepatitis or leptospirosis as the causative pathogens, and is in general not mentioned in the classical clinical picture of an HFRS case and especially not in PUUV. Actually, it has been suggested that liver involvement could be used as one of the key differentiators between SEOV infection and other hantavirus infections.[40,41] In the clinical cohort studies higher numbers of patients with proteinuria, liver injury and a longer febrile period have been reported in SEOV cases. However, one should take into consideration that the PUUV and SEOV clinical studies were performed in different populations, namely Western-European and Asian cohorts.

Presenting symptoms outside the classical HFRS triad are increasingly being reported in the literature and could lead to 'missed' cases and subsequent underdiagnosis.[7] Furthermore, recent papers debate the absolute difference between HFRS and HCPS hantavirus syndromes. It seemed in many cases that symptoms overlap and HFRS cases presented with acute respiratory failure without signs of kidney involvement while HCPS patients may show renal complications.[42] Therefore, it has been suggested to use the term 'hantavirus disease' for all hantavirus-related described syndromes.

CLINICAL MANAGEMENT AND TREATMENT

In both HFRS caused by SEOV and in HFRS/nephropathia epidemica due to a PUUV infection the initiation of prompt and proper supportive treatment is crucial, such as monitoring fluid balance, diuresis, kidney function and the use of fresh frozen plasma/transfusions in case of haemorrhagic complications when necessary.[32,43] Small trials and case reports have shown that ribavirin treatment can be useful in the very early phase of HFRS by reducing the risk of haemorrhagic events and the severity of renal insufficiency.[43-46] Interferon inhibits hantavirus replication *in vitro* but shows no beneficial effect *in vivo*, and the same holds true for adjunctive prednisolone treatment which showed no beneficial outcome in a placebo-controlled clinical trial.[2,43,44,47] Recently, two case reports described

efficient treatment of severe PUUV cases in Finland with the bradykinin receptor antagonist icatibant.[48,49] Since treatment and supportive care in PUUV and SEOV are the same, the importance for clinicians to differentiate between the two infections lies in the clinical course of infection and prognosis. Since both the haemorrhagic complications and CFR are much higher in SEOV, when compared with PUUV, clinicians might tend to provide an early start of ribavirin treatment in SEOV HFRS cases, where in PUUV the relative course of disease might not outweigh the side effects of ribavirin treatment.

DIFFERENTIAL DIAGNOSIS

As for most viral haemorrhagic fever pathogens, HFRS caused by SEOV has a broad differential diagnosis. Especially early in disease when symptoms are most likely to be aspecific it is impossible to differentiate between other viral or bacterial infections purely on a clinical basis. The broad differential diagnosis of an acute SEOV infection includes acute kidney injury, acute abdomen, septicaemia and more specifically leptospirosis, scrub typhus, murine typhus, dengue, haemorrhagic scarlet fever and the spotted fevers.[50] Considering that the distinction between HFRS and HCPS might not be as clear as historically thought, one should keep in mind that the differential diagnosis of more aspecific presentations of HFRS warrants a much broader differential diagnosis and subsequent diagnostic approach.

DIAGNOSTICS

The current diagnostics of HFRS in the Netherlands (and the majority of North-Western Europe where only PUUV and TULV are known to circulate)[51] relies on the basic assumption that PUUV is the causative agent. HFRS (nephropathia epidemica) by PUUV is routinely diagnosed by serology, as the viraemic stage is short and diagnostic requests for HFRS are often too late in the course of disease to justify diagnostics by reverse transcriptase-polymerase chain reaction (RT-PCR). However, the extent of viraemia varies per hantavirus species. In nephropathia epidemica, the level of viraemia is considerably lower than in more severe forms of HFRS as caused by SEOV.[4] As a consequence infection with SEOV might provide a broader time window for molecular detection than PUUV. For PUUV the optimal timeframe for molecular detection lies within the first four days of onset of illness,[4,52,53] while for SEOV routine molecular detection has been described up to eight days post onset of disease,[24,28,38,52,53] with one report even mentioning molecular detection in the second week.[28] Therefore, molecular testing for HFRS using a genus-wide or PUUV/ SEOV multiplex RT-PCR on samples taken up to eight days after onset needs to be considered in countries with known circulation of both PUUV and SEOV.

Since almost all acute cases of HFRS have IgM and IgG antibodies against the nucleocapsid protein of hantaviruses, serodiagnostics are the most commonly used method for verifying hantavirus infection using indirect IgG and IgM enzyme-linked immunosorbent assays (ELISA), IgM capture ELISAs or immunofluorescence assays (IFA).[54,55] However, routine hantavirus serology targeting PUUV as causative agent might encounter problems with the ready detection of antibodies specific to SEOV. Although cross-reactivity exists in hantavirus serology, PUUV and SEOV are in different cross-reacting serogroups reflecting the relatedness of their carrier rodents. PUUV antibodies show the strongest cross-reaction with TULV and New World hantaviruses such as Sin-Nombre virus (SNV) while SEOV antibodies

Table 1. *Comparison between Puumala hantavirus and Seoul hantavirus infection in humans. Based on references*[28,35,47,51]

Characteristics	PUUV	SEOV
Carrier	*Myodes glareolus* (bank vole)	*Rattus norvegicus, R. rattus* (brown, black rat)
Geographic distribution	Europe	Worldwide (recently emerging in Western Europe)
Syndrome	Mild HFRS (NE)	Moderate HFRS
Incubation period	2-3 weeks	2-3 weeks
Peak viraemia	Up to 4 days post onset of symptoms	Up to 8 days post onset of symptoms
CFR	<0.1%	1-2%
Petechiae	12%	25-30%
Haemorrhagic complications	2-5%	Up to 50%
Leucocytosis	23-57%	72%
Elevated transaminases	41-60%	>80%
Requiring dialysis	5-7%	25-30%
Nausea-vomiting	33-83%	Up to 100%
Myopia/ blurred vision	10-36%	15-20%
Melaena	~10%	~20%

show strong cross-reactivity with the genotypes of DOBV/SAAV and HNTV. Between these two groups the cross-reactivity is weak or sometimes completely absent.[2,51] The weak cross-reactivity in the serological response between the two groups is reflected in the test specifications for some commercial PUUV-specific ELISAs and IFAs which report a (strongly) reduced diagnostic efficiency for SEOV and are therefore not indicated for the diagnosis of HFRS caused by SEOV. Genus-wide ELISA methods using a cocktail of antigens from different hantavirus species might address this issue. However, commercially available tests show a very low sensitivity for SEOV (as low as 50%) which will result in missed cases as well. In addition, there are mosaic IFA slides on the market which offer parallel, multiplex testing for IgM and IgG antibodies to PUUV, DOBV/SAAV (the two most common genotypes), SEOV, HTNV and SNV. According to the test specifications, the cumulative specificity and sensitivity for infection with a hantavirus are excellent, including the diagnostic efficiency for PUUV. However, the efficiency to pinpoint an HFRS case to a SEOV infection seems less optimal. Countries that have endemic circulation of both DOBV/SAAV and PUUV (North-Eastern Germany, Central Europe)[50] will have covered both serogroups in their routine diagnostics, which might suffice for routine SEOV diagnostics. There are commercial ELISAs based on HTNV antigens that are offered for both DOBV/SAAV and HTNV/SEOV diagnostics. Finally, because of the observed cross-reactivity in hantavirus serological responses, comparative virus neutralisation tests remain the gold standard in hantavirus serology to confirm an infection with a specific hantavirus species.[2,7] However, as the neutralising antibodies are not always virus species-specific early in infection (probably due to both neutralising IgM and IgA), virus neutralisation is only indicated in later phases of infection and when definitive insight into the causative hantavirus species is wanted. It is important that the adequacy for SEOV diagnostics of the numerous hantavirus serology tests available on the market is evaluated in routine diagnostic settings for their performance in SEOV diagnostics.

CONCLUDING REMARKS

The presence of SEOV in rat populations in the Netherlands has direct implications for the clinician and routine hantavirus diagnostics. The clinical outcome of an infection with SEOV and PUUV will greatly benefit from an early diagnosis which will reduce the costs of unnecessary tests and treatments as well. This can be secured by increased awareness among physicians for both mild and moderate HFRS and the availability of diagnostics properly validated for both PUUV and SEOV. Laboratories performing hantavirus diagnostics in countries were SEOV emerges should review and revalidate their current hantavirus diagnostics (targeting PUUV and/or DOBV), for adequate diagnosis of SEOV infection.

DISCLOSURES

This study was partially funded by EU grant FP7-602525 Prepare and FP7-261504 EDENext and is catalogued by the EDENext Steering Committee as EDENext 308 (http://www.edenext.eu).

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ORIGINAL ARTICLE

Identifying targets for quality improvement in hospital antibiotic prescribing

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ABSTRACT

Objectives: To audit antibiotic use in a university hospital and to identify targets for quality improvement in a setting with low antibiotic use and resistance rates.

Methodology: A point-prevalence survey (PPS), using a patient-based audit tool for antibiotic use, was executed in the Radboud University Medical Centre in May 2013. On one index day, all patients on systemic antibiotics hospitalised > 24 hours were included. Data regarding antibiotic prescriptions were extracted from the medical records. Multiple logistic regression analysis was performed in order to predict whether a variable was associated with low guideline compliance or a low rate of consulting an infectious disease specialist.

Results: 428 hospitalised patients were included, of whom 40.9% received antibiotics.

Overall, 75.7% of all prescriptions were compliant with the guidelines in place and for 87.8% the reason for prescription was documented. Amoxicillin/clavulanic acid (OR = 4.08, 95% CI 1.57-10.56), and respiratory tract infections (RTI) (OR = 6.17, 95% CI 2.55-14.94) were associated with low compliance with guidelines. An infectious disease physician or medical microbiologist was less often consulted for empirical therapy (OR 23.21, 95% CI 6.37-84.51) or empirical therapy continued > 72 hours (OR 14.69, 95% CI 3.56-60.56) compared with prescriptions that were based on culture results. In addition, fewer consultations were requested for RTI (OR 4.47, 95% CI 1.39-14.35).

Conclusion: A PPS is a good tool to identify targets for antibiotic stewardship in routine clinical practice. Several areas for improvement, such as a low compliance with guidelines for amoxicillin/clavulanic acid and RTI, and a low rate of consulting an infectious disease physician or medical microbiologist concerning antibiotic therapy in case of RTI and empirical therapy continued > 72 hours were identified.

KEYWORDS

Antibiotic prescription, drug utilisation, antibiotic stewardship, point prevalence survey, Netherlands

INTRODUCTION

The causal relation between the development of antibiotic resistance and the use of antibiotics is well accepted and multifactorial. In the hospital setting as well as the outpatient setting, unnecessary or inappropriate antibiotic prescribing is common.[1-3]

To curb the development of antibiotic resistance and reduce the unnecessary use of antibiotics, the Dutch Working Party on Antibiotic Policy (SWAB) recently developed a viewpoint document, which describes the antimicrobial stewardship policy needed for Dutch hospitals. To establish an efficient antimicrobial stewardship program in the hospital, the first step is to identify current institutional resistance patterns and antibiotic usage rates. In addition, it is important to identify targets for quality improvement. Not every hospital requires the same level of intervention and, therefore, an institutional stewardship program should be tailored for each hospital to their own problem pathogens and local overuse of particular classes of drugs in specific patient groups or treatment indications.[4]

In order to obtain a clear overview of hospital antibiotic prescribing at the patient level, the European Centre for Disease Prevention and Control (ECDC) has developed a standardised data collection technique at the patient level to measure total antibiotic consumption, including indications and method of use. The aim of a point-prevalence survey (PPS), according to the ECDC, is to provide a relatively fast and cheap way to evaluate total antibiotic use, and indications for prescription.[5]

Publications of a single-centre PPS presenting results of antibiotic consumption are scarce, especially in a setting with low antibiotic consumption and low antibacterial resistance rate. The objectives of this study were to obtain an inventory of antibiotic use in a university hospital and to identify targets for quality improvement of prescription by using a PPS.

METHODS

Design and setting

A point-prevalence study was performed at the Radboud University Medical Centre Nijmegen (Radboudumc, 953 beds). This is one of the largest hospitals in the Netherlands, providing supra-regional tertiary care for a population ~2.5 million residents of the south-eastern part of the country.

Antimicrobial guidelines for the most common infectious diseases were introduced in the hospital more than 30 years ago, and have been revised at regular intervals. Antimicrobial guidelines in the Radboudumc are, except for small revisions regarding local restrictions, based on the guidelines developed by the SWAB (http://www.swab.nl). At present, the electronic version of the antimicrobial guidelines in the hospital is easily accessible on the internet, and available on every computer in the Radboudumc. At the time of this study, the overall antibiotic consumption for the Radboudumc was 65.2 defined daily dose/100 bed-days.

For this study, no approval of the local medical ethics committee (CMO Arnhem-Nijmegen) was required, since it was part of quality control of drug utilisation, observational in nature, data used for this study were already available in the electronic patient records, and data were processed anonymously. Department heads were notified by a letter, explaining the aim of the study.

Data collection

A PPS, using the ECDC patient-based audit tool for antibiotic consumption as guideline, was completed on 15 May 2013. For all patients who were on antibiotics at the time of the survey and admitted to the hospital for > 24 hours, data were collected through screening of medical records by a multidisciplinary team, comprising infectious disease specialists (IDS: medical microbiologists/infectious disease physician) and hospital pharmacists. Whenever the reason for treatment was not documented, the attending physicians were contacted in order to obtain missing information. To be included in the study, patients had to be admitted to the hospital for at least 24 hours and still be present at 08.00 a.m. on the day of the study. If patients were temporarily away from the wards for diagnostics and procedures, or at home for a few hours, they were still included. Outpatients, patients in day care (e.g. haemodialysis), and patients in psychiatric wards were excluded. Antibiotics belonging to group J01 (antibacterials for systemic use) of the Anatomic Therapeutic Chemical (ATC) classification system from the World Health Organisation (WHO) Collaborating Centre for Drug Statistics Methodology were included in the study.[6] Rifamycin (J04AB03) was included if used for infections other than those caused by mycobacteria. When multiple antibiotics had been prescribed to a patient, all antibiotics were recorded. Antifungal or antiviral therapies, as well as antibiotics used for non-systemic selective digestive tract decontamination, and antibiotics used as prokinetics were excluded.

Optimal antibiotic use and standards for prophylaxis were based on the hospital guidelines. Guideline compliance can be defined as prescribing the correct antibiotic, according to the hospital guidelines, and assessment was done based only on the information written in the patient records. Guideline compliance was classified into four categories: (I) according to hospital guidelines, (II) recommended by an IDS, (III) according to ward-specific policies, (IV) other (i.e. recommendation of supervisor; international consensus). Categories I, II, and III were considered to be compliant with the guidelines, while category IV was considered non-compliant, unless deviations were based on valid grounds and no other recommendation was available. A more detailed description on data collection can be found in the supplementary data.

Data analysis and statistics

Data were entered into a database, double checked by the investigator, and analysed using IBM SPSS statistics version 21.0. Thirty-one wards were aggregated into three main specialities: medicine (including: internal medicine, oncology, haematology, nephrology, gastro-enterology, neurology, cardiology, pulmonology, urology, gynaecology, paediatrics and geriatrics), surgery (including: neurosurgery, orthopaedics, cardiothoracic surgery, surgery, otorhinolaryngology, oral surgery, and obstetrics), and intensive care units (ICU) (including: intensive and medium care, and neonatal intensive care).

Standard frequency tabs were used to present the main results and antibiotics are presented at the WHO ATC-5 level.[6] Continuous variables are expressed as proportions. Categorical variables are expressed as proportions and were analysed by Fisher's exact test or Pearson's chi-square test where applicable. Logistic regression analysis was performed in order to determine whether or not a variable was associated with guideline compliance or rate of consulting an IDS. Univariable logistic regression analysis was used to assess the relationship of each potential predictor with the outcome measure. Variables were included in the multivariable logistic regression analysis based on a p-value

≤ 0.25 in the univariable analysis. Using a backward and a stepwise forward approach for the iterative process of variable selection, variables were removed from the model if they were considered not statistically significant at a p-value ≤ 0.05. The probability of effect modification was controlled by adding interaction terms to the multivariable logistic regression analysis. A variable was considered to be an effect modifier when the interaction term turned out to be significant at p ≤ 0.1. The predicting accuracy and variation of the models was estimated by using the Hosmer and Lemeshow test and the Nagelkerke Pseudo R^2. For the Hosmer and Lemeshow test, poor fit was indicated by p < 0.05. The Nagelkerke Pseudo R^2 provides an indication of the amount of variation in the dependent variable explained by the model; since we used many questionnaires, a variation percentage < 10% was considered as low.

RESULTS

Overview of prescribing

A total of 428 hospitalised patients were evaluated, of whom 175 (40.9%) were prescribed 230 antibiotics, representing 1.31 courses per patient. More than one agent was prescribed to 25.7% of all patients who were on antibiotics. Data for proportions of treated patients, proportion of patients receiving multiple antibiotics, parenteral route of administration, inclusion of start and reason in patient record for the different main specialities are summarised in *table 1*.

Overall, 63.9% of antibiotics were administered via the parenteral route. Large differences in the proportion of parenteral use were found among departments, with a significantly higher intravenous rate (p = 0.001) in the ICU (85.3%) compared with medical (55.9%) and surgery (71.7%) departments.

The reason was documented in medical records for the majority (87.8%) of antibiotic prescriptions. There was no significant difference in documentation rate between specialities. However, the documentation rate was associated with the indication for prescription of antibiotics. Surgical prophylaxis had a significantly higher documentation rate (100%, p = 0.01), compared with medical prophylaxis (79.5%) and therapeutic antibiotics (86.5%).

Of the 141 prescriptions that concerned antibiotics for therapeutic use, 70.2% (n = 99) were prescribed for empirical therapy, of which 29.3% (n = 29) were prolonged after 72 hours of treatment in the presence of a negative culture result, or in the absence of culture samples. Only 42 prescriptions were directed at a known pathogen. The top 5 sites of infection accounted for > 60% of all

Table 1. *Overview of prescribing stratified for main specialities*

	Specialities			
Variable	**ICU**	**Medicine**	**Surgery**	**All**
Number of included patients	68	242	118	428
Number of patients with HAI (%)	12 (17.6)	26 (10.7)	14 (11.9)	52 (12.1)
Number of patients with CAI (%)	12 (17.6)	82 (33.8)	29 (24.6)	123 (28.7)
Number of patients receiving antibiotics (%)	24 (35.3)	108 (44.6)	43 (36.4)	175 (40.9)
Number of prescriptions	34	143	53	230
Mean courses per patient	1.42	1.32	1.23	1.31
Multiple antibiotics (%) 2 agents (%) 3-4 agents (%)	 6 (25.0) 2 (8.3)	 22 (20.4) 6 (5.5)	 8 (18.6) 1 (2.3)	 36 (20.6) 9 (5.1)
Route of administration Parenteral (%) Oral (%) Inhalation (%)	 29 (85.3) 4 (11.8) 1 (2.9)	 80 (55.9) 62 (43.4) 1 (0.7)	 38 (71.7) 14 (26.4) -	 147 (63.9) 80 (34.8) 2 (0.9)
Indication for prescription Infection (%) Medical prophylaxis (%) Surgical prophylaxis (%)	 26 (76.5) 4 (11.8) 4 (11.8)	 84 (58.7) 40 (28.0) 19 (13.3)	 31 (58.5) - 22 (41.5)	 141 (61.3) 44 (19.1) 45 (19.6)
Start and reason of antibiotic documented (%)	27 (79.4)	128 (89.5)	47 (88.7)	202 (87.8)

HAI = hospital-acquired infection; CAI = community-acquired infection.

therapeutic prescriptions. Respiratory tract infections (RTI) (18.4%; n = 26) and skin, soft tissue, bone and joint infections (18.4%; n = 26) were most common. Other common infections were urinary tract infections (12.8%; n = 18), infections of the central nervous system (8.5%; n = 12), and intra-abdominal infections (7.8%; n = 11).

Forty-five prescriptions were for surgical prophylaxis, of which 6.7% (n = 3) were prolonged for more than 1 day. Forty-four prescriptions were issued for medical prophylaxis, mainly for haematology and oncology patients. An overview of the antibiotics prescribed, stratified for indications, can be found in the supplementary data.

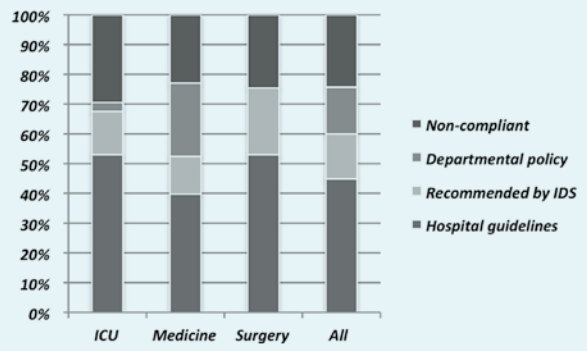
Guideline compliance

Guideline compliance was calculated by the sum of prescriptions compliant with hospital guidelines (44.8%), prescriptions according to departmental guidelines which are not approved by the hospital antibiotic committee (15.7%) and prescriptions recommended by an IDS (15.2%), resulting in an overall compliance rate of 75.7%.

Medicine departments prescribed significantly more often according to guidelines used on wards ($p < 0.001$) compared with surgical departments and the ICU. No differences between the main specialities were found for prescribing according to hospital guidelines or based on the recommendations of an IDS. *Figure 1* presents an overview of reasons for choosing a particular antibiotic stratified by main specialities.

Table 2 shows an overview of the univariable regression analysis including all potential predictors assessed, and the final multivariable regression analysis predicting non-compliance to guidelines. The strongest predictor for non-compliance was RTI (26 prescriptions were indicated for RTI, non-compliance 61.5%, crude OR 6.56, 95% CI 2.77-15.54, OR adjusted for amoxicillin/clavulanic acid 6.17, 95% CI 2.55-14.94). Prescription of amoxicillin/clavulanic acid (22 prescriptions, non-compliance 54.5%, five amoxicillin/clavulanic acid prescriptions were indicated for RTI, non-compliance 60.0%), recorded an OR of 4.08 (95% CI 1.57-10.56). Further analysis of data did not reveal an association between the use of amoxicillin/clavulanic acid and treatment of RTI for any specific speciality (data not shown).

Figure 1. *Overview of reasons for choosing a particular antibiotic, stratified by main specialities*



Consultation of infectious disease specialist

An IDS was involved in 76 (33.0%) of all prescriptions for antibiotic therapy, mainly in response to culture results (n = 49; 64.5%). No significant differences in consulting rate were found between the three main specialities.

Table 3 shows an overview of the univariable regression analysis including all potential predictors assessed, and the multivariable regression analysis predicting not consulting an IDS.

Indication for prescription was the strongest predictor for consulting an IDS. Physicians less often consulted an IDS for empirical therapy (OR 23.21; 95% CI 6.37-84.51) or prolonged empirical therapy (OR 14.69; 95% CI 3.56-60.56) compared with consultation for prescriptions that were based on culture results. In addition, fewer consultations were requested for RTI (OR 4.47; 95% CI 1.39-14.35).

Time investment

Data were collected by a multidisciplinary team of 15 persons, with an average time investment of 1.5 hours per person. Because of the advanced electronic patient records used by Radboudumc it was possible to see at a glance whether or not a patient was on antibiotics. In addition, practically all information could be found in the patient records. If, accidentally, the reason for prescription was not documented or if the reason for antibiotic choice was not according to hospital guidelines or on advice of an IDS, the attending physician was consulted by the team member during their weekly consultation at the department.

Preparation ahead of the PPS, writing a protocol and developing registration forms both tailored to the hospital, instruction for data collection team, time needed for planning and informing departments was estimated at 16 hours. Time required for data preparation and data analysis took a further estimated 16 hours.

DISCUSSION

This PPS provides a clear overview of total antibiotic use and the indications for prescriptions in a tertiary care centre. Of 428 patients included, 40.9% received antibiotics, which is in line with recent prevalences reported for other Dutch tertiary care hospitals.[7] The overall guideline compliance in this study was 75.7%, and amongst the highest reported by other studies in the field (range 52.4-70.0%).[8-10] However, there was a wide range in compliance rate between departments.

Table 2. *Univariable and multivariable analysis predicting non-compliance to guidelines*

	Univariable analysis				Multivariable analysis		
	OR	**95% CI**	**P-value**		**OR**	**95% CI**	**P-value**
Speciality				Speciality			
Surgery	1		0.74	Surgery	-	-	-
ICU	1.28	0.49-3.37	0.61	ICU	-	-	-
Medicine	0.92	0.44-1.93	0.83	Medicine	-	-	-
Restricted antibiotics (yes vs. no)				Restricted antibiotics (yes vs. no)			
Use of co-amoxiclav	4.47	1.81-11.03	0.001	Use of co-amoxiclav	4.08	1.57-10.56	0.004
Use of meropenem	1.04	0.20-5.29	0.96	Use of meropenem	-	-	-
Use of ciprofloxacin	1.77	0.67-4.68	0.25	Use of ciprofloxacin	-	-	-
Use of piperacillin-tazobactam	3.46	1.23-9.96	0.018	Use of piperacillin- tazobactam	-	-	-
Site of infection (yes vs. no)				Site of infection (yes vs. no)			
SSTBJ	0.92	0.35-2.43	0.87	SSTBJ	-	-	-
RTI	6.56	2.77-15.54	<0.001	RTI	6.17	2.55-14.94	<0.001
CNS	0.61	0.13-2.86	0.53	CNS	-	-	-
UTI	0.81	0.26-2.56	0.73	UTI	-	-	-
IA	1.17	0.30-4.59	0.82	IA	-	-	-
Hospital-acquired infection (yes vs. no)				Hospital-acquired infection (yes vs. no)			
Yes	0.49	0.24-1.03	0.06	Yes	-	-	-
Indication for prescription	1		0.26	Indication for prescription	-	-	-
Surgical prophylaxis	1.59	0.55-4.66	0.39	Infection	-	-	-
Medical prophylaxis	2.08	0.85-5.04	0.11	Surgical prophylaxis	-	-	-
Infection				Medical prophylaxis			

OR = odds ratio; SSTBJ = skin soft tissue bone and joint infections; RTI = respiratory tract infections; CNS = infections of the central nervous system; UTI = urinary tract infections; IA = intra-abdominal infections. Goodness of fit for multivariate analysis Hosmer and Lemeshow test 0.53, variation Nagelkerke Pseudo R^2 0.17.

Several targets for improving the quality of antibiotic use were identified in this study. The low guideline compliance for the treatment of RTI was surprising, in particular the high use of amoxicillin/clavulanic acid. The low rate of consulting an IDS in case of RTI may explain this, and at the same time indicates a mode for improvement. This applies to prescribing empirical therapy for > 72 hours in the presence of a negative culture result or in the absence of culture samples obtained, even though, with this definition, a few patients (e.g. patients with community-acquired pneumonia who cannot produce sputum) might be misclassified. This should be analysed in more depth in future surveys. The deviation from hospital guidelines and frequent use of departmental protocols in oncology and haematology departments is remarkable and another target for intervention, either to integrate in hospital general guidelines as far as these protocols are evidence-based or otherwise to exclude from further use.

The fact that the use of amoxicillin/clavulanic acid was significantly associated with low guideline compliance might indicate that additional measures, such as pre-authorisation or post-prescription review, are desirable. It has been demonstrated that non-guideline compliance often leads to the use of more broad-spectrum antibiotics.[11] Prescribers should be more aware that a more broad-spectrum empirical therapy does not result in more effective treatment, but does increase the selection of antibiotic resistance.

Empirical therapy should be de-escalated to culture results whenever possible. The large number of empirical therapies that were not de-escalated within 72 hours, when culture results became available, is another area with room for improvement. In these cases, it was less likely that an IDS had been consulted, and such consultation may help to improve outcome.

The overall percentage of hospital-acquired infection (HAI) reported in this survey (12.7%) is higher than percentages reported for tertiary care hospitals in other European studies (range 4.0%-9.7%).[5,7] As expected, the ICU had the highest proportion of patients treated for HAI. However, 22% of all patients with HAI were transferred from other hospitals and might have acquired the infection earlier. Therefore, the prevalence of HAI in a tertiary care hospital may not necessarily reflect the local risk of infection.

Table 3. *Univariable and multivariable analysis predicting not consulting an IDS*

	Univariable analysis				Multivariable analysis		
	OR	**95% CI**	**P-value**		**OR**	**95% CI**	**P-value**
Speciality				Speciality			
Surgery	1		0.59	Surgery	-	-	-
ICU	0.67	0.23-1.92	0.45	ICU	-	-	-
Medicine	1.07	0.47-2.43	0.88	Medicine	-	-	-
Restricted antibiotics (yes vs. no)				Restricted antibiotics (yes vs. no)			
Use of co-amoxiclav	2.23	0.77-6.39	0.14	Use of co-amoxiclav	-	-	-
Use of meropenem	0.35	0.07-1.79	0.21	Use of meropenem	-	-	-
Use of ciprofloxacin	2.33	0.56-9.70	0.25	Use of ciprofloxacin	-	-	-
Use of piperacillin-tazobactam	3.44	1.04-11.38	0.04	Use of piperacillin-tazobactam	-	-	-
Site of infection (yes vs. no)				Site of infection (yes vs. no)			
SSTBJ	0.20	0.07-0.58	0.03	SSTBJ	-	-	-
RTI	6.30	2.22-17.89	<0.001	RTI	4.47	1.39-14.35	0.01
CNS	0.77	0.23-2.56	0.67	CNS	-	-	-
UTI	0.51	0.18-1.44	0.20	UT	-	-	-
IA	1.36	0.39-4.67	0.63	IA	-	-	-
Hospital acquired infection (yes vs. no)				Hospital acquired infection (yes vs. no)			
Yes	1.15	0.61-2.15	0.66	Yes	-	-	-
Indication for prescription				Indication for prescription			
Directed to known	1		<0.001	Directed to known	1		<0.001
pathogen	26.56		<0.001	pathogen	23.21	6.37-84.51	<0.001
Empirical	18.42	7.42-95.15	<0.001	Empirical	14.69	3.56-60.56	<0.001
Empirical >72 h		4.61-73.75		Empirical >72 h			

OR = odds ratio; SSTBJ = skin soft tissue bone and joint infections; RTI = respiratory tract infections; CNS = Infections of the central nervous system; UTI = urinary tract infections; IA = intra-abdominal infections. Goodness of fit for multivariate analysis Hosmer and Lemeshow test 0.94, variation Nagelkerke Pseudo R^2 0.42.

Overall, and as reported in other European and national studies, β-lactams are the most frequently prescribed class of antibiotics.[10,12] The use of combinations of penicillins with enzyme inhibitors, third-generation cephalosporins, fluoroquinolones and carbapenems was found to be in line with the proportions reported for university hospitals in Nethmap 2013, the national surveillance report on antimicrobial use and resistance in the Netherlands.[12]

For 87.8% of cases, the reason for prescription was documented, which was found to be in line with documentation rates reported in other European studies (range 27.2%-92.3%).[9,10,13,14] However, these numbers indicate that there is still room for improvement. Documentation of start and reasons in the patient record is associated with more appropriate antibiotic prescribing,[15,16] and therefore, ECDC has identified this parameter as a key performance indicator.[5]

This study has several weaknesses. First, as in any PPS, this study was cross-sectional and thus only presents a snapshot of prescribing. Therefore, repeated PPS or follow-ups are necessary in order to identify patterns in prescriptions, seasonal variation and to assess changes in prescribing behaviour.

Secondly, based on the data collected, no statement could be made on whether or not patients who received parenteral antibiotics where eligible for a parenteral-oral switch of antibiotics. It is therefore recommended for future studies to add the item whether or not a patient is eligible for a parenteral-oral switch at the moment of the PPS.

In conclusion, the present study revealed several areas of practice that deserve specific attention in an institutional antimicrobial stewardship program. The most important targets identified are the low guideline compliance rate for amoxicillin/clavulanic acid and RTI, the frequent use of departmental protocols other than hospital antibiotic guidelines, and the large number of prescriptions for RTI and for prolonged empirical therapy without consulting an IDS. Despite the fact that a PPS only presents a snapshot of prescribing, it is helpful in setting priorities for quality improvement of antibiotic prescribing. Repeated PPS are necessary to reveal patterns in antibiotic prescribing and

to evaluate the effectiveness of interventions, and it is recommended to perform a PPS at least once a year.

ACKNOWLEDGMENTS

The authors thank E. Kolwijck, I. Maat, S. Debaest, J. van Ingen, I. Lede, J. van Pelt, B. Lestrade, R. van Crevel, B. Rodenburg, J. Kleinnijenhuis, R. Brüggemann, E. van der Wouden, N. van Erp, E. Muilwijk, B. Jacobs, K. de Kanter for their participation in data collection and T. Sprong, Canisius Wilhelmina Hospital, Nijmegen for his assistance in setting up the PPS.

DISCLOSURES

The authors declare no conflicts of interest. No funding or financial support was received.

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SUPPLEMENTARY DATA

Detailed description of data collection

Standardised case record forms were used for data collection, which included details on the number of inpatients in each department, patient age and gender, surgery in the past 30 days, transfers from other hospitals or nursing homes, antibiotics prescribed, route of administration, indication for prescribing (i.e. empirical, prolonged empirical (> 72 hours), medical prophylaxis or surgical prophylaxis), type of infection (i.e. hospital-acquired infection (HAI) or community-acquired infection (CAI)), site of infection, adherence to guidelines, documentation of reason for prescription in notes, and documented consultation of an infectious disease specialist (IDS).

The term 'prescription' was used to indicate every time an individual antibiotic was prescribed. Prescriptions were defined as either prophylactic or therapeutic. Antibiotics prescribed without clinical evidence of infection, and without a statement in the electronic patient record indicating a specific suspected infection were considered prophylaxis.[1] Prophylactic antibiotics were defined as a prescription in order to prevent the patient from acquiring an infection, and could either be surgical prophylaxis to prevent postoperative infections, or medical prophylaxis to prevent infections in the immunocompromised patient. Antibiotic therapy, prescribed before culture results were known, was considered to be empiric therapy. Empiric therapy used > 72 hours in the presence of a negative culture result or in the absence of culture samples was defined as prolonged empiric therapy.[1]

In the Radboudumc, restricted release of antimicrobial agents has been implemented as a method to improve antibiotic prescription. The restricted-release antibiotics include piperacillin-tazobactam, carbapenems, third- and fourth-generation cephalosporins, glycolpeptides and aminoglycosides. At the time of the study, the Radboudumc antibiotic committee considered adding amoxicillin-clavulanic acid (co-amoxyclav) to this list of restricted release antibiotics.

Consultation by an IDS was classified as: (I) initiated by the IDS in response to culture results, (II) initiated by the attending physician in response to culture results, (III) initiated by the attending physician, not in response to culture results.

Table 1. *Overview of prescribed antibiotics stratified by main indications for use*

Agent	All indications n %		Infection n %		Surgical prophylaxis n %		Medical prophylaxis n %	
Cefazolin	30	13.0	1	0.7	29	64.4	-	-
Co-trimoxazole	27	11.7	3	2.1	-	-	24	54.4
Co-amoxiclav	22	9.6	17	12.1	5	11.1	-	-
Ceftriaxone	21	9.1	19	13.5	1	2.2	1	2.3
Ciprofloxacin	20	8.7	9	6.4	-	-	11	25.0
Piperacillin-tazobactam	16	7.0	15	10.6	1	2.2	-	-
Flucloxacillin	15	6.5	15	10.6	-	-	-	-
Ceftazidime	14	6.1	14	9.9	-	-	-	-
Metronidazole	13	5.7	8	5.7	5	11.1	-	-
Amoxicillin	8	3.5	8	5.7	-	-	-	-
Meropenem	8	3.5	8	5.7	-	-	-	-
Teicoplanin	5	2.2	5	3.5	-	-	-	-
Colistin	4	1.7	2	1.4	-	-	2	4.5
Trimethoprim	4	1.7	-	-	-	-	4	9.1
Azithromycin	3	1.3	1	0.7	-	-	2	4.5
Cefuroxime	3	1.3	-	-	3	6.7	-	-
Clindamycin	3	1.3	3	2.1	-	-	-	-
Nitrofurantoin	3	1.3	3	2.1	-	-	-	-
Benzathine benzylpenicillin	2	0.9	2	1.4	-	-	-	-
Gentamicin	2	0.9	2	1.4	-	-	-	-
Rifampicin	2	0.9	2	1.4	-	-	-	-
Daptomycin	1	0.4	1	0.7	-	-	-	-
Erythromycin	1	0.4	1	0.7	-	-	-	-
Moxyfloxacin	1	0.4	1	0.7	-	-	-	-
Piperacillin	1	0.4	-	-	1	2.2	-	-
Vancomycin	1	0.4	1	0.7	-	-	-	-
Total	230	100.0	141	100.0	45	100.0	44	100.0

Infections could be either a hospital-acquired or a community-acquired infection. CAI were defined as cases where symptoms or antibiotics were started < 48 hours after a patient was admitted or > 30 days after discharge or surgery, while HAI were defined as cases where symptoms started > 48 hours after admission to hospital or < 30 days after surgery or discharge.[2] HAI were classified into four categories: (I) post-intervention (e.g. ventilator-associated pneumonia, intravenous catheter or urinary catheter), (II) postoperative, (III) *Clostridium difficile*-associated diarrhoea, and (IV) other HAI.

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ORIGINAL ARTICLE

Burden of highly resistant microorganisms in a Dutch intensive care unit

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ABSTRACT

Background: The occurrence of highly resistant microorganisms (HRMOs) is a major threat to critical care patients, leading to worse outcomes, need for isolation measures, and demand for second-line or rescue antibiotics. The aim of this study was to quantify the burden of HRMOs in an intensive care unit (ICU) for adult patients in a university hospital in the Netherlands. We evaluated local distribution of different HRMO categories and proportion of ICU-imported versus ICU-acquired HRMOs. Outcome of HRMO-positive patients versus controls was compared.

Methods: In this prospective single-centre study, culture results of all ICU patients during a four-month period were recorded, as well as APACHE scores, ICU mortality and length of stay (LOS) in the ICU.

Results: 58 of 962 (6.0%) patients were HRMO positive during ICU stay. The majority (60%) of those patients were HRMO positive on ICU admission. HRMO-positive patients had significantly higher APACHE scores, longer LOS and higher mortality compared with controls.

Conclusions: Our study suggests that a large part of antibiotic resistance in the ICU is imported. This underscores the importance of a robust surveillance and infection control program throughout the hospital, and implies that better recognition of those at risk for HRMO carriage before ICU admission may be worthwhile. Only a small minority of patients with HRMO at admission did not have any known risk factors for HRMO.

KEYWORDS

Critical care, highly resistant microorganisms, HRMO, length of stay, surveillance, isolation

INTRODUCTION

Antibiotic resistance in the critical care population is an ever-increasing problem.[1] The high use of antimicrobial therapy in the intensive care unit (ICU),[2] the large number of invasive procedures, the density of a susceptible patient population, the severity of underlying illness, and flaws in infection control measures are all contributing factors resulting in ICUs as 'epicentres' of antimicrobial resistance in hospitals.[3,4] ICUs are considered generators of antimicrobial resistance.[3] In addition to acquisition of HRMOs in the ICU, part of the resistance problem is imported to the ICU through already colonised or infected patients admitted from other hospitals, general wards, or from the community.[5]

This continuous and rising threat of antimicrobial resistance is of relevance considering the outcome in patients infected with resistant rather than susceptible microorganisms is worse.[6] Measures to prevent cross-contamination include surveillance, barrier precautions and antibiotic stewardship. All preventive measures are labour intensive, costly and some are patient unfriendly. Resistance to first-line antibiotics urges the use of 'rescue' or second-line antibiotics with little hope of new effective alternatives in the near future.[7]

The incidence and characteristics of resistance can vary widely depending on local circumstances. According to European surveillance data, the Netherlands, along with Scandinavian countries, is considered an area with low incidence of antimicrobial resistance for Gram-positive as well as Gram-negative bacteria.[8] However, even in the Netherlands, prevalence of antimicrobial resistance in the community is not negligible, and is emerging.[9-11]

We evaluated the incidence of HRMOs in our ICU to quantify the total burden of HRMOs, to clarify the local distribution of different categories of HRMOs and the proportion of imported versus acquired HRMOs in our

ICU. Furthermore, we evaluated the outcome of patients affected by colonisation or infection with any HRMO vs. controls in terms of ICU mortality and length of stay (LOS) in the ICU. Finally, we wanted to characterise this subpopulation colonised or infected with an HRMO to enable better *a priori* recognition of affected patients, thus rendering better opportunities for adequate treatment and infection control.

MATERIALS AND METHODS

This is a single-centre study involving prospective data collection from 1 October 2009 to 31 January 2010 in an academic teaching hospital with 40 critical care beds distributed over 4 units (medical, cardiothoracic, neurological, and general surgery). All four units consist of a large multi-bed floor combined with a few rooms for isolation. On the floors, standard hygienic procedures are maintained. Annually, between 2500 and 3000 patients are admitted. All patients admitted in the four-month study period were included for analysis. The study was approved by the ethics committee and the requirement of informed consent was waived. We recorded baseline characteristics including sex, age, referring speciality, unit of admission, APACHE II score, date and source of admission (emergency department or general ward vs. other hospital). APACHE II score was not recorded for cardiosurgical patients because this score is not validated for this subgroup. Of note, patients from various sources were admitted to the four separate units, e.g. the cardiothoracic unit did not only admit cardiothoracic surgery patients but other patients as well. Patients were considered referred from another hospital if they were transferred either directly to the ICU or indirectly through another ward in our hospital. The vast majority of this last group were admitted to the general ward for less than a week before admission to our ICU.

All cultures taken either by indication or in the context of our structured surveillance program were evaluated. Surveillance screening included cultures from throat, nose, rectum, sputum and urine on admission, followed by cultures from throat, nose, rectum and sputum on day four and twice weekly thereafter during the stay in the ICU. Surveillance cultures were obtained from those patients with an anticipated stay of 48 hours or more on the day of admission. Patients referred from elsewhere were included in surveillance screening on the day of admission regardless of anticipated or actual length of stay.

Culture results were retrieved from the database of the department of medical microbiology. Susceptibility testing was done according to European guidelines (European Committee on Antimicrobial Susceptibility Testing, EUCAST).

HRMOs were defined by criteria issued by the Dutch Working Party on Infection Prevention *(table 1)*.[12] All patients colonised or infected with an HRMO were placed in full contact isolation, as dictated by our protocol for infection prevention. A patient could be included only once in the study group; subsequent readmissions of the same patient were excluded from the study group but were analysed nevertheless. Only the first positive culture for any HRMO in an individual was recorded; subsequent cultures with the same organism were regarded as the same event. Different species of HRMOs within one patient were recorded as separate events. No distinction was made between either colonisation or infection with an HRMO. Of the patients with an HRMO, further details such as antibiotic use during the hospital stay and medical history were retrieved from the patient's file. Other outcome measures for the entire study population included ICU mortality and LOS on the ICU.

We tried to identify clusters of HRMOs by analysing whether identical species of HRMOs were cultured in different patients during their ICU stay on the same unit.

Statistical methods included the χ^2-test, Fisher's exact test, Student's t-test, Mann-Whitney U-test and Wilcoxon rank test, where appropriate, using Minitab® Release 14.1 and Graph Pad Prism (Prism 5 for Windows, version 5.04, Nov 6 2010) software.

RESULTS

A total of 1061 admissions were recorded, 91 of which were re-admissions within the study period; hence 962 admitted patients were included in the study population (*table 2*). Baseline characteristics are presented in *table 2 and 3*. In 58 (6.0%) patients an HRMO was found (in total 60 HRMOs; two patients had two different HRMOs). For distribution of HRMO species we refer to *table 4*. The distribution of these 58 patients according to unit and patient category is depicted in *table 2*.

Of 232 patients (24.1%) referred from another hospital, 16 patients were colonised with an HRMO during their stay in our ICU (6.9%), compared with 42 out of 730 patients (5.8%) referred from our hospital (p = 0.52).

In those patients with any HRMO (n = 58), 47 patients (82.8%) were found to have an HRMO within the first three days of ICU stay. Of these, 11 (23.4%) were referred from another hospital: 36 (76.6%) were admitted from a general ward of this hospital or from the emergency department.

Of those not referred from elsewhere and found positive for an HRMO within three days (n = 36), 32 patients (88.9%) had one or more comorbid conditions (*table 4*). In this group of 36 patients, 27 patients (75.0%) had been admitted in the three months preceding current admission

Table 1. *Definition of highly resistant microorganisms (HRMOs)*[12]

	ESBL	Quino-lones	Amino -glycosides	Carba-penems	Co-trimo-xazole	Ceftazi-dime	Pipera-cillin	Penicil-lins	Glyco-peptides	Oxacillin	Methi-cillin
Enterobacteriaceae											
E. coli	A	B	B	A	--	--	--	--	--	--	--
Klebsiella spp	A	B	B	A	--	--	--	--	--	--	--
Other	A	B	B	A	B	--	--	--	--	--	--
Non-fermenting gram-negative											
Acinetobacter spp.	--	B	B	A	--	B	--	--	--	--	--
Stenotro-phomonas spp.					A		--	--	--	--	--
Other		C	C	C		C	C	--	--	--	--
Gram-positive											
S. pneumoniae	--	--	--	--	--	--	--	A	A	--	--
Enterococcus spp.	--	--	--	--	--	--	--	B	B	--	--
S. aureus	--	--	--	--	--		--	--	--	A	A

Resistance to one antibacterial agent in category A, to ≥ two in category B, or ≥ three in category C required to define microorganism as highly resistant microorganism (HRMO). ESBL = extended beta-lactamase (resistance to any third-generation cephalosporin used as proxy in *E. coli, Klebsiella* spp., and *Proteus* spp.)

Table 2. *Patient characteristics*

	Total	Without HRMO	With HRMO	P
Study population, n	962	904	58	
Male n (%)	595 (61.9%)	556 (61.5%)	39 (67.2%)	0.38 (NS)
Age, years, median (range)	62 (12-91)	63 (12-91)	58 (16-82)	0.22 (NS)
APACHE II score, median (range)(n)** • APACHE II > 20 (n =101) • APACHE II ≤ 20 (n = 431)	13 (2 – 52) (532)	13 (2-44) (488) 83 (17.0% of 488) 405	18 (2-52) (44) 18 (40.9% of 44) 26	< 0.001 < 0.001
Unit, n (%) • Cardiopulmonary unit • Surgical unit • Medical unit • Neurosurgical unit	 390 (40.5%) 238 (24.7%) 181 (18.8%) 153 (15.9%)	 381 (42.1%) 211 (23.3%) 168 (18.6%) 144 (15.9%)	 9 (15.5%) 27 (46.6%) 13 (22.4%) 9 (15.5%)	< 0.001
Patient category, n (% of total) • Cardiopulmonary surgery • Medical • Surgical • Neurosurgical • Trauma • Neurological • Gynaecological	 405 (42.1%) 208 (21.6%) 181 (18.8%) 103 (10.7%) 44 (4.6%) 17 (1.8%) 4 (0.4%)	 392 (43.4%) 186 (20.6%) 167 (18.5%) 101 (11.2%) 38 (4.2%) 16 (1.8%) 4 (0.4%)	 13 (22.4%) 22 (37.9%) 14 (24.1%) 2 (3.4%) 6 (10.3%) 1 (1.7%) 0 (0%)	

**APACHE II-score available for 532 (95.5%) of non-cardiosurgical patients; HRMO = highly resistant microorganism.

Table 3. *Patient characteristics and outcome*

	Total	Without HRMO	With HRMO	P
Study population, n (%)	962	904 (94.0%)	58 (6.0%)	
Referral from other hospital, patients, n (%)	232 (of 962; 24.1%)	216 (of 904; 23.9%)	16 (of 58; 27.6%)	0.52 (NS)
Admitted through general ward or emergency department, n (%)	730 (of 962; 75.9%)	688 (of 904; 76.1%)	42 (of 58; 72.4%)	0.84 (NS)
Positive blood cultures, patients, n‡‡	28	25	3 (HRMO *E.coli* 2, MRSA 1)	
LOS ICU, days, median (range)	1 (1-130)	1 (1 - 88)	5 (1 – 130)	< 0.001
ICU mortality, patients, n (%)	74 (7.7%)	63 (7.0%)	11 (19.0%)	0.0031

‡‡ Blood cultures with common skin contaminants (e.g. coagulase-negative Staphylococci, viridans group Streptococci) had to be cultured on two or more separate occasions to be included (n = 23 cultures with positive culture with skin contaminant on one occasion excluded); HRMO = highly resistant microorganism; LOS = length of stay.

Table 4. *HRMO species and patient characteristics with HRMO*

HRMO, patients, n (% of total patients)	**58 (6.0%)**
HRMO, total, n*	60
Enterobacteriaceae	50
E.coli	40
Klebsiella spp.	2
Other†	8
Non-fermenting gram-negatives	5
Pseudomonas spp.	4
Other‡	1
Gram-positives	5
VRE	3
MRSA	2
LOS ICU on first day of positive HRMO culture	
Days, median (range)	1 (1-77)
1 day, n (% of 60)	36 (60%)
2- 7 days, n (% of 60)	12 (20%)
8-14 days, n (% of 60)	4 (6.7%)
> 14 days, n (% of 60)	8 (13.3%)
HRMO patients not referred from elsewhere and HRMO within three days (% of 58)	36 (62.1%)
Admitted in preceding 3 months (n,% of 36)	27 (75.0%)
Recent antibiotic exposure (n,% of 36)	12 (33.0%)
Comorbid conditions (n,% of 36)	32 (88.9%)
Cardiovascular (n,% of 36)	11 (30.6%)
Malignancy (n,% of 36)	10 (27.8%)
Organ transplantation (n,% of 36)	7 (19.4%)
Pulmonary (n,% of 36)	6 (16.7%)
Diabetes (n,% of 36)	4 (11.1%)
Chronic hepatitis (HCV, HBV††) (n,% of 36)	2 (5.6%)
Occupational exposure (pig farmer) (n,% of 36)	2 (5.6%)
No known risk factor for HRMO (n,% of 36)	2 (5.6%)

*2 patients had 2 HRMOs. †*E. cloacae* 4; *Citrobacter* spp. 3; *S. marcescens* 1. ‡*S. paucimobilis* 1; HRMO = highly resistant microorganism; LOS = length of stay; HBV = hepatitis B virus; HCV = hepatitis C virus.

to the ICU; 12 patients (33.3%) had received antibiotics in the months preceding ICU admission. Two patients (of 36, 5.6%) were farmers working with livestock (pigs); both were found to be methicillin-resistant *Staphylococcus aureus* (MRSA) positive. Two (of 36, 5.6%) had no comorbid conditions, no recent hospital admission, no recent antibiotic treatment and no occupational exposure to HRMOs.

Median LOS for all ICU patients was 1 day (range 1-130 days, mean 4 days). LOS in the ICU for HRMO-positive patients was significantly longer (median 5 days, range 1-130, mean 16 days) compared with HRMO-negative patients (median 1 day, range 1-88, mean 3 days) (p = 0.000) (*table 3*).

LOS in the ICU at the time of first positive culture for any HRMO was 1 day in 36 (60%), 2-7 days in 12 (20%), 8-14 days in 4 (7%) and more than 14 days in 8 (13%) (*table 4*).

Patient categories with most HRMO-positive patients were medical (22 out of 208, 10.6%), surgical (14 out of 181, 7.7%) and trauma (6 out of 42, 13.6%). Units with most HRMO-positive patients were the surgical unit (27 out of 218, 11.3%) and the medical unit (13 out of 181, 7.2%).

Of patients admitted to our ICU for more than 14 days, 18 of 57 (31.6%) were found to have any HRMO during ICU stay vs. 31 of 840 (3.7%) patients staying 7 days or less in our ICU (p < 0.0001).

APACHE II score for HRMO-positive patients (available in 57 patients) was significantly higher (median 17, mean 19, range 2-52) compared with the APACHE II score for HRMO-negative patients (available in 875 patients) (median 13, mean 13, range, 2-44) (p = 0.000).

Overall ICU mortality was 74 (7.7%); mortality was significantly higher in patients with HRMO (11 out of 58,

19.0%) than patients without HRMO (63 out of 904, 7.0%) (p = 0.0031) (*table 3*).

Further, 25 patients had a positive blood culture with a susceptible microorganism and three other patients had a positive blood culture with an HRMO (*E. coli* 2, MRSA 1).

In the readmitted (excluded) patient group (n = 99), 16 patients (16.2%) had any HRMO (*E. coli* 6, *Klebsiella* spp. 3, other Enterobacteriacea 3, *Pseudomonas* spp. 1, other non-fermenting Gram-negatives 1, vancomycin-resistant Enterococci 3). This percentage of HRMO-positive patients is significantly higher compared with the percentage of HRMO-positive patients in the study group (6.0%, p = 0.0002)

In this study, we could not identify patient characteristics with sufficient specificity and sensitivity to predict HRMO carriage. During the study period, we did not find clusters of identical HRMOs indicating an outbreak.

DISCUSSION

In this single-centre prospective study on the burden of HRMOs in critical care patients in an area where HRMOs are non-endemic,[8,13] it is an important finding that more than half of HRMO-positive patients were identified from cultures taken on admission. This finding suggests that an important part of antibacterial resistance is imported to the ICU, rather than acquired during the ICU stay. Indeed, hospitalisation on a general ward prior to ICU admission is a recognised risk factor for HRMO acquisition[14] and although the proportion of HRMOs introduced onto the ICU through already colonised or infected patients has been quantified in studies for MRSA,[15] its contribution for all HRMOs has, to the best of our knowledge, not been clearly elucidated as yet in our region. Of all patients admitted through the emergency department or general ward, 36 (out of total 932, 3.7%) had an HRMO within three days of ICU stay. Two (of 36, 5.6%) of these patients had no comorbid conditions, no recent hospital admission, no recent antibiotic treatment and no occupational exposure to HRMOs. Although a minority, this underscores the fact that HRMO is not restricted to the hospital, even in our area of low antibiotic resistance. Indeed, prevalence of antimicrobial resistance in the community, for instance carriage of extended-spectrum beta-lactamases-producing Enterobacteriaceae, is considerable, where contribution of contaminated foods – mainly poultry – and travel, remains to be elucidated.[9-11]

Only three patients had a proven infection (bacteraemia) with any HRMO, therefore colonisation appears to be far more frequent than a serious infection. That HRMO-positive patients have a higher mortality might in part be explained by the fact that sicker patients are more often colonised with any HRMO. Indeed, in our study population those with an HRMO had a significantly higher APACHE-II score than those without HRMO (*table 2*).

In our cohort, Gram-negative bacteria comprised the largest part of all HRMOs. This is in line with the trend towards more Gram-negative antibiotic resistance in European ICUs, with a stabilisation or decrease in Gram-positive antibiotic resistance.[1,16] Recently, others described cephalosporin and aminoglycoside resistance in a substantial number of critically ill patients colonised with Enterobacteriaceae (15 and 10%, respectively) on ICU admission in a large Dutch multi-centre trial.[17]

We could not identify clusters of HRMO. Therefore, the hygiene measures set forth to contain the spread of HRMOs appeared sufficient in this study period.

Our finding that a substantial part of HRMOs are imported into the ICU underscores the imperative need to ensure strict application of hygienic practices, such as hand washing, as well as excellent use of antibiotic stewardship throughout medical care, inside and outside the hospital. Furthermore, this finding highlights the importance of a conscientious surveillance program on the ICU. Along this line, surveillance screening *before* possible ICU admission in specific populations on medical and surgical wards, or in patients with a high risk of community-acquired HRMO, could be worthwhile in order to prevent cross-contamination on these wards and on the ICU. Indeed, in the Netherlands, a surveillance program comparable to our practice on the ICU is carried out on haemato-oncology and dialysis wards as well. Likewise, it is common practice to screen those with occupational exposure to livestock known to have a high carriage rate of MRSA prior to or at hospital admission.[18] Expanding surveillance to other high-risk populations, for instance those admitted to a surgical ward for an extended period, especially when receiving antibiotics, might be beneficial. Some variables have been recognised as risk factors for carriage of HRMOs upon ICU admission, such as prior antimicrobial treatment, prior hospitalisation, and residence in a nursing home.[5] However, due to sample size, we could not extract patient characteristics from our study population to predict HRMO carriage. It might be helpful to further characterise those patients at risk for HRMO carriage in the context of a larger epidemiological study. This could lead to more differentiated isolation procedures and a more sophisticated choice of antibiotics in case of a proven or suspected infection. Further studies, however, do necessitate a uniform definition of 'highly resistant' enabling meaningful comparisons and data aggregation; currently, a wealth of definitions are being applied in the literature.[19]

There are some limitations to our study, the most important being that it is a single-centre study with a relatively small sample size. It is important to note that

patients with an intended stay of two days or less are not included in the surveillance program, unless transferred from another hospital; positive cultures could thus have been missed in these patients. In this group of patients with a short LOS in the ICU, these potential false-negatives would contribute to our finding that a substantial number of HRMO-positive patients are found to be as such in the first days of admission. As our surveillance program is robustly implemented in our daily practice, it is unlikely that patients were missed out of this program for procedural reasons. Some patients underwent surveillance screening despite a short stay of 48 hours or less on the ICU. We did not differentiate between colonisation and infection with any HRMO. Causality between occurrence of an infection with an HRMO and worse outcome in the HRMO group can thus not be proven in this observational study. We did not analyse all known risk factors for HRMO carriage (referral from a nursing home was not recorded); due to sample size, subgroup analyses were not feasible.
It could be of benefit to further characterise those at risk of harbouring infection or colonisation with an HRMO, using a uniform international definition of HRMO, with expanding surveillance in high-risk groups outside the ICU, in order to enable maximum precautionary measures and give optimal antibiotic treatment. This combination of surveillance and timely isolation can prevent further spread of HRMOs, our biggest challenge in infection control in critically ill patients in the years ahead.

CONCLUSION

This observational study suggests that HRMOs on the ICU are quite often imported and not only acquired during the stay in the ICU. Gram-negative HRMOs were more abundant than Gram-positive and are of clinical significance even in a non-endemic area. Although most patients with any HRMO had comorbid conditions, were recently admitted to a hospital, had received antibiotics prior to ICU admission, or had occupational exposure to an HRMO, a small minority had no relevant history. Our findings underscore the importance of infection control and optimal surveillance on admission to the ICU.

ACKNOWLEDGEMENTS

We would like to thank Mr. M. Lazonder for building the database and Mr. H. van Assen for providing APACHE II scores of included patients. Furthermore, we wish to thank Ms. K. Glatman for correcting the English text.

DISCLOSURES

The authors declare no conflicts of interest. No funding or financial support was received.

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ORIGINAL ARTICLE

Serum kisspeptin levels across different phases of the menstrual cycle and their correlation with serum oestradiol

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ABSTRACT

Objective: A rise in oestrogen in the preovulatory phase produces a GnRH-induced luteinising hormone surge. Oestrogen receptors are not found on GnRH neurons but these are present on kisspeptin neurons. That led us to hypothesise that serum kisspeptin levels may vary during various phases of the menstrual cycle in relation to serum oestradiol.

Methods: Thirty female students, 18-25 years old, Saudi nationality, with a regular menstrual cycle, were recruited from various health colleges of the University of Dammam, Saudi Arabia. Three blood samples per volunteer were collected at three different times: the early follicular, preovulatory and luteal phase. Serum kisspeptin and oestradiol were measured using ELISA kits. Comparison between individual subjects during the various phases was done by one-way, repeated-measures ANOVA. To discover which specific means differed, Bonferroni post hoc test was applied. Pearson's correlation was used to find out the relationship.

Results: There was a statistically significant increase ($p < 0.001$) in serum kisspeptin levels from the early follicular to the preovulatory phase (264.11±28.42 vs. 472.46±17.82 nmol/l respectively), and from the preovulatory to the luteal phase (472.46±17.82 vs. 724.79±36.85 nmol/l respectively). Oestradiol levels also increased significantly ($p = 0.006$) from the early follicular to the preovulatory phase (45.85±5.34 vs. 79.07±7.45 pg/ml respectively), Pearson's correlation revealed a statistically insignificant correlation between kisspeptin and oestradiol in all three phases.

Conclusion: Endogenous kisspeptin secretion seems to vary across the different phases of the menstrual cycle and is not related to serum oestradiol.

KEYWORDS

Kisspeptin, oestradiol, menstrual cycle

INTRODUCTION

Kisspeptin, discovered in 1996 in the context of tumour pathophysiology,[1] is a powerful stimulator of the reproductive system. It plays a central role in influencing the timing of puberty.[2] Kisspeptin is believed to be responsible for the two modes of gonadotropin-releasing hormone (GnRH) secretion: the oestrogen-induced ovulatory surge of GnRH/luteinising hormone (LH) and basal, pulsatile GnRH/LH release.[3,4] Within the hypothalamus, GnRH neurons are situated very close to the kisspeptin neurons and they express kisspeptin receptor as well.[5,6] Kisspeptin is believed to mediate gonadal steroid feedback to the hypothalamus. Although androgens, oestrogen and progesterone suppress gonadotropin secretion, none of these sex steroids affect GnRH secretion by direct action on GnRH neurons due to the absence of their receptors on GnRH neurons.[7]

Recent evidence suggests that kisspeptin neurons may act as main upstream regulators that integrate central and peripheral signals, hence causing the release of GnRH from GnRH neurons.[8-10]

In a female monthly cycle, just before ovulation, there is a rise in serum oestrogen which causes a rise in GnRH resulting in an LH surge. Surprisingly, GnRH neurons do not have oestrogen receptors[7] whereas kisspeptin neurons do have oestrogen receptors.[11]

Keeping in mind the oestrogen-induced preovulatory LH surge, lack of oestrogen receptors in GnRH neurons, and their presence on kisspeptin neurons, we hypothesised that serum kisspeptin levels may vary during the various phases of the menstrual cycle and variation in these levels may have some role in ovulation. So far, virtually all research on kisspeptin signalling has focused on exogenous kisspeptin administration and its effects on reproduction. None of the studies measured fluctuations in endogenous kisspeptin secretion with a changing sex-steroid milieu. This compelled us to design the present

project and to determine serum kisspeptin levels in various stages of the menstrual cycle in Saudi female students.

MATERIAL AND METHODS

Permission and ethical approval for this study were sought from the deanship of Scientific Research, University of Dammam, Saudi Arabia. Thirty female students from various colleges within our university were recruited. The study participants met the following inclusion criteria: 18-25 years old Saudi females, with a regular menstrual cycle (cycle length varying between 25 and 35 days with no more than 5 day variability), no use of prescription medications (including hormonal contraception) for at least 2 months before the study, and willing to participate. Exclusion criteria were the presence of a chronic medical condition, irregular menstruation, and inaccessibility to follow-up.

The subjects were briefed about the project in a familiarisation session. They agreed to inform us on the first day of their next cycle to plan the dates for the blood sampling. The individual length of each participant's menstrual cycle was considered when scheduling their appointments for the experiments. Three blood samples per volunteer were collected at three different times.[12]

1) Early follicular phase: During days 2-5 from the onset of the menstrual cycle.

2) Preovulatory phase: During 11-16 days before the onset of the next menstrual cycle.

3) Luteal phase: 3-5 days before the onset of the next menstrual cycle.

Verification of the menstrual cycle phase was done by basal body temperature (BBT, high oestrogen during the follicular phase lowers BBT; high progesterone after ovulation raises BBT)[12] and serum oestradiol levels.[13-15]

All blood samples were obtained by venipuncture, after an overnight fast between 8-10 a.m. (in order to minimise the effect of circadian rhythm on kisspeptin levels). These samples were allowed to clot and centrifuged within 30 minutes after venipuncture. The serum obtained was frozen at -80 °C until further analysis by ELISA kits for measurement of serum kisspeptin and oestradiol.

STATISTICAL ANALYSIS

Statistical analysis was done by SPSS version 20. Mean, maximum, minimum and standard deviation were calculated by descriptive statistics. Comparison between individual subjects during the follicular, luteal and ovulatory phase was done by one-way, repeated-measures ANOVA. In case of data violation of the assumption of sphericity, we used Greenhouse-Geisser values. To discover which specific means differed, the Bonferroni post hoc test was applied. Pearson's correlation was used to find out the relationship. For all tests, the level of significance was set at $p < 0.05$.

RESULTS

The mean duration of the menstrual cycle in all study subjects was 29±1 (mean ± SD) days (27-31 days). The duration of the menses was 6±1 days (mean ± SD). All participants recruited had, according to their past history, a regular menstrual cycle. Serum kisspeptin and oestradiol levels were different in the different phases of the menstrual cycle (*table 1*). Maximum concentrations of oestradiol and kisspeptin were observed in the preovulatory and luteal phase, respectively. The repeated measures ANOVA with a Greenhouse-Geisser correction revealed that mean serum kisspeptin differed statistically significantly between time points/phases ($f = 61.524$, $p = 0.000$). Post hoc testing using the Bonferroni correction revealed that there was a statistically significant increase ($p = 0.000$) in serum kisspeptin levels from the early follicular to the preovulatory phase (264.11±28.42 vs. 472.46±17.82 nmol/l respectively), and from the preovulatory to the luteal phase (472.46±17.82 vs. 724.79±36.85 nmol/l respectively) (*table 1*). Therefore, we can conclude that serum kisspeptin levels change statistically significantly across these three phases of menstruation.

The repeated measures ANOVA with a Greenhouse-Geisser correction determined that mean serum oestradiol also differed statistically significantly between time points/phases ($f = 6.632$, $p = 0.004$). Post hoc testing using the Bonferroni correction revealed that there was a statistically significant ($p = 0.006$) increase in serum oestradiol levels from the early follicular to the preovulatory phase (45.85±5.34 vs. 79.07±7.45 pg/ml respectively), but statistically insignificant ($p = 1.000$) decrease from the preovulatory to the luteal phase (79.07±7.45 vs. 68.95±6.41 pg/ml respectively) (*table 1*).

No correlation was found between serum kisspeptin and oestradiol in the follicular, preovulatory and luteal phase.

DISCUSSION

The present study revealed endogenous kisspeptin secretion in the various phases of the menstrual cycle in young female participants with regular menstruation. To our knowledge, this is the first analysis of its type. We did not find any association between serum kisspeptin levels and body mass index; a finding in accordance with a recent study in which no remarkable changes in kisspeptin

Table 1. *Serum kisspeptin and serum oestradiol in various stages of menstrual cycle*

Parameters	Early follicular phase (Mean ± SEM)	Preovulatory phase (Mean ± SEM)	Luteal phase (Mean ± SEM)	p value*	p value**	
					FP versus OP	OP versus LP
Serum kisspeptin (nmol/l)	264.11±28.42	472.46±17.82	724.79±36.85	0.000	0.000	0.000
Serum oestradiol (pg/ml)	45.85±5.34	79.07+-7.45	68.95±6.41	0.004	0.006	1.000

*p value was determined with one-way, repeated-measures ANOVA; **P value was determined by Bonferroni (95% confidence interval); FP = early follicular phase; OP = preovulatory phase; LP = luteal phase.

expression were found in diet-induced obese experimental rats compared with controls.[16] Serum concentrations of kisspeptin and oestradiol both increased statistically significantly in the preovulatory phase when compared with the early follicular phase (*table 1*). Hence, our study indicates that as the sex-steroid milieu (oestrogen and progesterone) fluctuates during the different phases of the female menstrual cycle, kisspeptin levels also fluctuate. Mammalian ovulation requires an LH surge brought about by the positive feedback action of oestradiol on GnRH release. High kisspeptin levels in the preovulatory phase suggest that kisspeptin might be responsible for the LH surge. Our results are in agreement with Smith *et al.*, who showed that kisspeptin expression increases just before ovulation and during a steroid-induced LH surge in ovariectomised rats.[17] Clarkson *et al.* also revealed that kisspeptin neurons become activated at the time of ovulation.[18] Some studies have shown that inhibition of kisspeptin action abolishes the pro-oestrous LH surge and inhibits oestrous cyclicity in rats.[19,20] Thus, kisspeptin signalling seems to be essential for the preovulatory GnRH/LH surge. As further proof in the role of kisspeptin in ovulation, recent evidence suggests that lactating rats have low levels of KiSS mRNA and protein in the hypothalamus.[21] Hence, the cause of increased kisspeptin in the ovulatory phase could be increased activation of kisspeptin neurons and increased expression induced by high oestradiol as documented in above-mentioned animal studies.

Few studies have measured the response to exogenous kisspeptin administration in terms of GnRH-stimulated LH release in varying sex-steroid milieus such as the follicular, preovulatory and luteal phase women.[22] Yee-Ming Chan detected that the response to kisspeptin (as measured by kisspeptin-induced, GnRH-induced LH pulse) was largest in preovulatory women, intermediate in luteal-phase women, and smallest in follicular-phase women.[22] Dhillo *et al.*[23] similarly observed the largest LH responses in preovulatory women after exogenous administration of kisspeptin compared with the follicular and luteal phases. Jayasena *et al.*[24] discovered that preovulatory women had a significantly large LH response to kisspeptin but early to mid-follicular-phase women did not. These findings are also concordant with studies in rats[10] and sheep[25] in which the largest LH response to kisspeptin was seen just before ovulation.

We also observed a significant increase in serum kisspeptin levels from the preovulatory to the luteal phase. At the moment we are unable to provide a rational clarification for this rise. However, as more and more avenues about kisspeptin are explored, we may come up with an appropriate justification in forthcoming years.

The serum oestradiol levels showed typical monthly variations, with the lowest oestradiol levels in early follicular, intermediate in luteal and peak levels in preovulatory phase, in accordance with previous research.[26,27] Our data also suggested that serum kisspeptin levels were not related to oestradiol in any of the stages. Further studies should be performed to explore the factors affecting serum kisspeptin levels.

CONCLUSION

By measuring serum kisspeptin levels in females during the different phases of the menstrual cycle and by relating these to oestradiol, we are beginning to gather insight into the fundamental physiology of kisspeptin across the human menstrual cycle. Our results suggest that endogenous kisspeptin secretion varies across the menstrual cycle. However, kisspeptin levels do not seem to be related to oestradiol in any of the stages. The physiological mechanisms underpinning these differences may be explored by prospective studies measuring kisspeptin under conditions where the sex-steroid milieu and other factors are directly controlled and manipulated.

STRENGTH AND LIMITATIONS, AND RECOMMENDATION

The strength of our study lies in its novelty, being the first one to explore kisspeptin fluctuations across the menstrual cycle. Although the sample size was relatively

small, limiting our ability to draw inferences, the data are important for potential further understanding of the role of kisspeptin in the female monthly cycle. We believe that to obtain a more precise depiction of kisspeptin, the sampling regime would need to be much more frequent, and more reliable. If the role of kisspeptin in ovulation is confirmed, it might offer a potential basis for a new infertility treatment.

DISCLOSURES

The authors acknowledge the financial support from Deanship of Scientific Research, University of Dammam, Saudi Arabia by grant number 2013134.

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CASE REPORT

A 'shocking' finish to the Dam tot Damloop event

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ABSTRACT

A patient with status epilepticus after long-distance running is described. The patient, a young woman, was brought to our hospital with status epilepticus after completing in a running event, probably caused by an extremely low phosphate level of 0.30 mmol/l. Hypophosphataemia is a rare complication of running and can be caused by the use of phosphate in the glycogenolytic and glycolytic pathway.

KEYWORDS

Exercise, hypophosphataemia, seizure

What was known on this topic?
Neurological dysfunction as a consequence of exercise-induced hypophosphataemia was previously reported in some case reports 20 years ago. The exact pathophysiology is not known.

What does this add?
Our manuscript gives a reminder of a potentially serious complication of a very popular sport, namely running. We also give our view on the pathophysiology of hypophosphataemia. We think that it is important to know about a potentially life-threating complication of a very popular form of exercise, which can easily be managed with intravenous suppletion of phosphate.

INTRODUCTION

Running is currently a very popular form of exercise. The 'Dam tot Damloop' is a popular running event, between Amsterdam and Zaandam, with a total distance of ten English miles. In 2013, there were approximately 65,000 runners, both professionals and amateurs. Unfortunately, each year 10 to 15 participants are admitted to the Emergency Department of the Zaans Medisch Centrum, the hospital close to the finish line, because of syncope. Most of these runners exhibit signs of exertional heat stroke, a potentially life-threating condition in need of acute medical attention. There are also some electrolyte disorders that occur as a result of running. Hyponatraemia is a frequently observed disorder, especially in long distance running. Last year the team on the emergency department was confronted with a less frequent problem caused by running, namely extreme hypophosphataemia. This condition can cause serious complications, such as status epilepticus.

CASE DESCRIPTION

A 32-year-old, well-trained female was admitted because of syncope nearby the finish line of her 10-mile run. The weather conditions on the day of the race included an average temperature of 16.7 °C. with a humidity of 93%. Her past medical history showed a conjunctival melanoma in 1999, which was cured with local excision and radiation with strontium. At presentation in the Emergency Department, 15 minutes later, she was sweating but had no sequelae from the episode of syncope. Her blood pressure was normal at 114/82 mmHg, with a pulse rate of 112 beats/minute, her rectal temperature was 37.8°C and her respiration rate was 20 breaths/minute. Almost directly after presentation, blood was drawn and intravenous fluid resuscitation was started. About 10 minutes later she lost consciousness, with clear epileptic movements of the eye lids and extremities. A seizure was

diagnosed and intravenous benzodiazepines were given, with only a partial and short-lasting effect. The patient was admitted to the intensive care unit and treated with phenytoin, sedation and ventilator support. Laboratory investigation of the blood directly drawn after arrival at the emergency department and before the epileptic activity, showed a serum creatinine of 113 µmol/l (normal 49-90 µmol/l), a maximum creatine kinase of 1695 U/l (normal 0-140 U/l), a corrected calcium of 2.06 mmol/l (normal 2.20-2.60 mmol/l) and a severe hypophosphataemia of 0.30 mmol/l (normal 0.70-1.40 mmol/l). Sodium level was 140 mmol/l (normal 135-145 mmol/l), magnesium 0.68 (normal 0.66-1.07) and potassium 3.8 mmol/l (normal 3.2-4.9 mmol/l). Arterial blood gas examination revealed a metabolic acidosis, with a pH of 7.31 (normal 7.35-7.45), pCO2 35 mmHg (normal 35-45 mmHg), bicarbonate 17.6 mmol/l (normal 22.0-26.0 mmol/l) and a lactate of 2.6 mmol/l (normal < 2.2 mmol). Intravenous administration of phosphate was initiated, and the phosphate level rose to 0.62 mmol/l 90 minutes later. Five hours after the first laboratory results the phosphate level normalised, with a value of 1.18 mmol/l. In total, 40 mmol of phosphate was administered. The next day she had completely recovered, without signs of epileptic activity. An MRI scan of the brain showed no abnormalities. An EEG showed no signs of epileptic activity. 25-OH-vitamin D and parathyroid hormone levels were normal. The phosphate level in her urine was 5 mmol/l (normal: 13-44 mmol/l). Three months later, her calcium and phosphate levels were normal. The discharge diagnosis was status epilepticus, caused by a severe exercise-induced hypophosphataemia.

DISCUSSION

The most common complication of running is exertional heat stroke, with a very high temperature and syncope as essential signs. In our case, exertional heat stroke as a cause of syncope or hypophosphataemia is very unlikely, in our opinion, because of her normal rectal body temperature, stable haemodynamics and completely normal mental status at presentation. Exertional heat stroke can cause hypophosphataemia, probably as a result of the accompanying respiratory alkalosis that stimulates the enzyme phosphofructokinase to add a phosphate molecule to fructose-6 phosphate, forming fructose-1,6-diphosphate, thereby trapping phosphate in the cell. But our patient did not have a respiratory alkalosis in her arterial blood gas drawn one minute after the start of the epileptic activity.

Hypophosphataemia without exertional heat stroke is a very rare complication of exertion, and only a few case reports have been described.[1] In one small study, participants with a syncope during a running event had a significantly lower serum phosphate compared with runners who completed the run without syncope. The condition and phosphate level of all of the participants with a syncope improved and normalised spontaneously without interventions.[2] The exact cause or pathophysiology is unknown, but there are some possible explanations.

Phosphate is a very important substance in the physiology of muscle contractions. The required energy for muscle contractions is derived from breakdown of adenosine triphosphate (ATP). The breakdown of ATP results in the formation of energy, adenosine diphosphate (ADP) and phosphate. Because the store of ATP is quickly depleted, there is also some energy stored in phosphocreatine. The enzyme creatine kinase can break down phosphocreatine and this results in the formation of ATP. More energy for muscle contractions needs to come from the breakdown of glycogen, stored in the muscle and liver, also known as glycogenolysis. The enzyme phosphorylase splits glucose from glycogen by adding a phosphate molecule, thereby forming glucose-1-phosphate. Both epinephrine and glucagon stimulate the enzyme phosphorylase.[3] When glucose enters the (muscle) cell, a phosphate molecule is attached to prevent the glucose from leaving the cell, so it can be used to generate energy. This is done by the enzyme glucokinase, and glucose-6-phosphate is formed. During glycolysis, glucose is further degraded to produce energy. First, two molecules of phosphate are invested to form glucose-6-phosphate and fructose-1,6-diphosphate respectively. This will be further degraded to pyruvic acid and acetyl-CoA, respectively; the latter is used in the Krebs cycle to produce energy.[4]

Given these biochemical reactions, there are some possible explanations for exercise-induced hypophosphataemia. First, there can be some temporary loss of phosphate

Table 1. *Case reports/studies with an exercise-induced hypophosphataemia*

Year of publication	Journal	Author	Number of patients	Phosphate level (average)	Neurological symptoms
1986	BMJ	Dale et al.	15	?	16
2011	Clin J Sport Med	Mohseni et al.	1	0.29 mmol/l	1

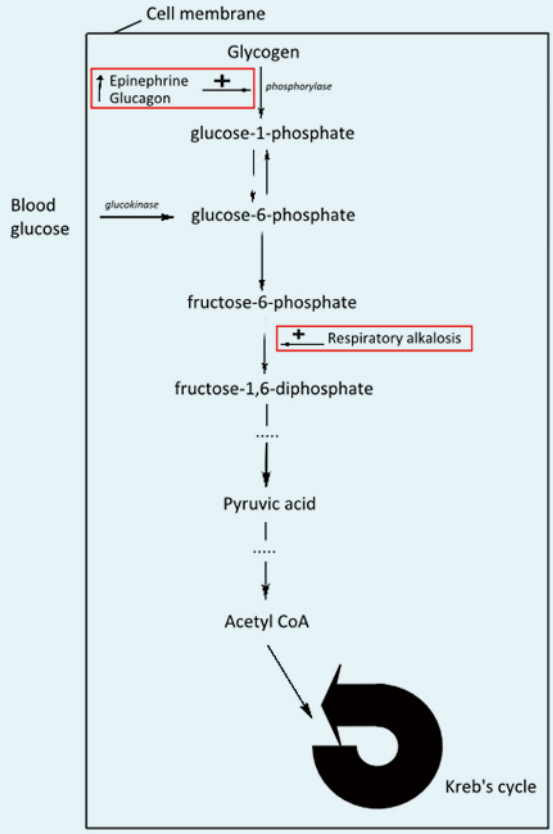
during the first steps of glycogenolysis and glycolysis, where phosphate is invested to form intermediate products in the process. On top of this, during exercise concentrations of epinephrine and glucagon will be higher, which will induce glycogenolysis by stimulating phosphorylase.[5] Otherwise, when glucose enters the cell during exercise, a phosphate molecule will be attached to prevent the exit of glucose out of the cell. Secondly, the depleted stores of phosphocreatine need to be replenished, with consumption of phosphate.[5]

In our case there was also a mild hypocalcaemia, which could be caused by sequestering of calcium into damaged muscles, but we believe the mild hypocalcaemia had no role in the neurological dysfunction.

Hypophosphataemia is related to different forms of neurological dysfunction, such as confusion, neuropathy, seizures and coma. The mechanism that leads to neurological dysfunction is unclear, but it can be caused by diminished levels of ATP and thus energy production in the central nervous tissue. Another reason can be lower concentrations of 2,3-diphosphoglycerate and ATP in red blood cells, causing an increased haemoglobin-oxygen affinity which in turn will cause lower oxygen delivery and oxygen concentration in nervous tissue cells.[3,6] This can also explain why our patient developed an epileptic state and other patients with a comparable phosphate level do not, because after running there is already diminished oxygen delivery to the brain as a result of an oxygen debt of the muscles.

The reason why some runners develop hypophosphataemia and others do not is unclear. A previous observational study showed a trend between the training status of runners and their phosphate levels after competition. It is unclear if this is caused by training, diet or just genetics.[7]

Figure 1. *Important steps of glycogenolysis and glycolysis*



CONCLUSION

In conclusion, syncope or neurological dysfunction during or directly after running is generally due to exertional heat stroke and some electrolyte disorders. A common electrolyte disorder is hyponatraemia. If the core temperature is completely normal, exercise-induced hypophosphataemia should be considered. We would like to alert you to the fact that hypophosphataemia could be a serious complication of exercise. It is important to recognise this condition, because it is easily treated and adequate treatment can prevent complications, although in one case report spontaneous normalisation of the phosphate levels occurred.[2]

DISCLOSURES

The authors declare no conflicts of interest. No funding or financial support was received.

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CASE REPORT

Successful treatment of fulminant postoperative bleeding due to acquired haemophilia

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ABSTRACT

Acquired haemophilia is a rare but life-threatening phenomenon in patients who have undergone surgical treatment. We describe a patient with a history of pancreatic cancer and a conventional pancreatico-duodenectomy, who underwent elective resection of an enterocutaneous fistula, complicated by fulminant haemorrhagic shock, caused by acquired haemophilia A. Eventually, the bleeding was controlled by a combination of aggressive haemostatic and immunosuppressive therapy. Prompt diagnosis of acquired haemophilia is crucial to allow early and appropriate haemostatic treatment and reduce the period of increased bleeding risk by eradicating the inhibitor with immunosuppressive therapy.

KEYWORDS

Haemophilia A, acquired, immunosuppressive agents, haemostasis

What was known on this topic?
Acquired haemophilia A is a rare cause of severe postoperative bleeding. Treatment consisted of haemostatic treatment and immunosuppressive therapy.

What does this add?
Delayed severe postoperative bleeding can be the manifestation of acquired haemophilia A, so prompt diagnosis is crucial to initiate the appropriate haemostatic and immunosuppressive treatment in order to reduce the period of severe bleeding which may affect outcome. The time for complete haemostasis to develop may be up to 14 days, so patience and supportive treatment in this period are necessary.

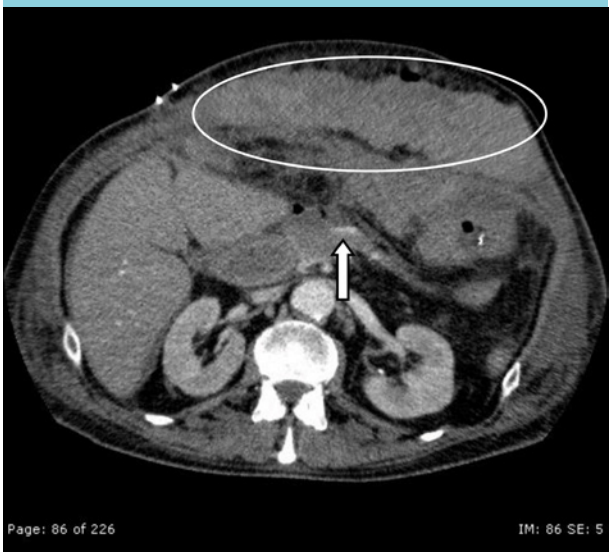
INTRODUCTION

Acquired haemophilia is a rare haemorrhagic disorder that results from the immune-mediated production of auto-antibodies to coagulation factors. The most common auto-antibodies that affect clotting factor activity and lead to a bleeding disorder are directed against and interfere with factor VIII (acquired haemophilia A). The reported incidence of this disease is approximately 1.5 per million.[1] The most common conditions associated with the development of acquired factor VIII inhibitors are rheumatic disease, malignancy, some drugs (penicillin, sulphonamides, phenytoin), and the postpartum period; however, most cases are idiopathic (approximately 52%).[2] Acquired haemophilia as a cause of post-procedural bleeding episodes is a potentially life-threatening disease in patients without a previous diagnosis of a bleeding disorder. Prompt diagnosis is crucial to initiate the appropriate treatment strategies based on the optimal haemostatic therapy and elimination of the inhibitor by immunosuppressive therapy. Here, we report a patient with delayed but profound postsurgical haemorrhage due to acquired haemophilia A, and illustrate that patience is necessary to achieve eradication of the auto-antibodies in order to achieve sustained control of the bleeding.

CASE REPORT

A 67-year-old man was diagnosed with pancreatic cancer for which he underwent a conventional pancreaticoduodenectomy (pT3N1M0, R0 resection), without receiving adjuvant systemic chemotherapy. The postoperative course was complicated by an enterocutaneous fistula, which was surgically resected two months after the first operation. Intraoperatively, there was an obvious oozing which was controlled by haemostatic gauzes. The early postoperative period was characterised by slowly decreasing haemoglobin levels. On the seventh postoperative day, the clinical condition of the patient abruptly deteriorated, characterised by circulatory shock. Computed tomography (CT) scan of the abdomen demonstrated a large mesenteric haematoma (*figure 1*), and active bleeding from one of the branches of the superior mesenteric artery, which was successfully embolised. Laboratory testing on the intensive care unit (ICU) revealed a haemoglobin level of 4.9 mmol/l, platelet count 375 x 10^9/l, a prothrombin time (PT) of 23 seconds, and an activated partial thromboplastin time (aPTT) of 99 seconds. Despite multiple transfusions (red blood cells, platelets, fresh frozen plasma, activated prothrombin complex concentrate, tranexamic acid, and vitamin K (*figure 2*)), the patient developed progressive circulatory shock. A second CT angiography showed a new blush in a distal branch of the right internal mammary artery which was also embolised. After that, an abdominal compartment syndrome developed, which required surgical decompression. Multiple transfusions of blood products and vitamin K normalised the PT; however, the aPTT remained prolonged. At this stage acquired haemophilia A was suspected and a mixing test was performed which confirmed the presence of an inhibitor. Further investigations showed a factor VIII activity of 8% (normal range 55-200%), and the Bethesda assay revealed a low titre of the present inhibitor against factor VIII (3.5 Bethesda units (BU)) (*table 1*). Factor VII activity (24%) was also reduced due to consumption caused by severe haemorrhage and a vitamin K deficiency. No inhibitor against factor VII was measured. Retrospectively, coagulation tests at the time of the pancreaticoduodenectomy showed normal clotting times. In consultation with the Van Creveld Clinic, the haemophilia treatment centre of the University Medical Center Utrecht, the patient was initially treated with factor VIII (AaFact® 50 IE/kg bolus, followed by 50 IE/kg/24 h). However, the suppletion of factor VIII was discontinued the next day due to a low yield in factor VIII activity (peak rise of 13%). Therefore, recombinant factor VIIa (R-FVIIa) (NovoSeven®) was started in a dose of 90 µg/kg (*figure 2*). To promote elimination of the factor VIII inhibitor, glucocorticoids were started (methylprednisone 1 gram/day for three days, prednisone 80 mg/day thereafter) plus cyclophosphamide (100 mg/day). After the start of immunosuppressive therapy, the patient also received prophylactic cotrimoxazole. Three days later (fourth laparotomy), no bleeding focus was detected and the gauzes were removed. An intra-abdominal drain nearby the recurrent leakage of the pancreaticojejunostomy was left behind. Five days after the start of immunosuppressive therapy, the patient became less dependent on red blood cell transfusions and the haemoglobin level remained stable. We continued R-FVIIa in tapering doses for three more days. At day 13, factor VIII had normalised, the antibody titre declined (1.2 U/ml), and approximately 30 days after the start of immunosuppressive therapy the aPTT normalised (*figure 3*). There were no recurrent episodes of haemorrhage and the prednisone dose was slowly tapered by 10 mg each week. Unfortunately, 35 days after admission to the ICU the patient developed a rapidly progressive abdominal septic shock due to ongoing leakage of the pancreaticojejunostomy causing multiple, non-resolvable, intra-abdominal abscesses. Despite administration of broad-spectrum antibiotics and supportive treatment, refractory septic shock developed and the patient died. The next of kin did not give permission for autopsy.

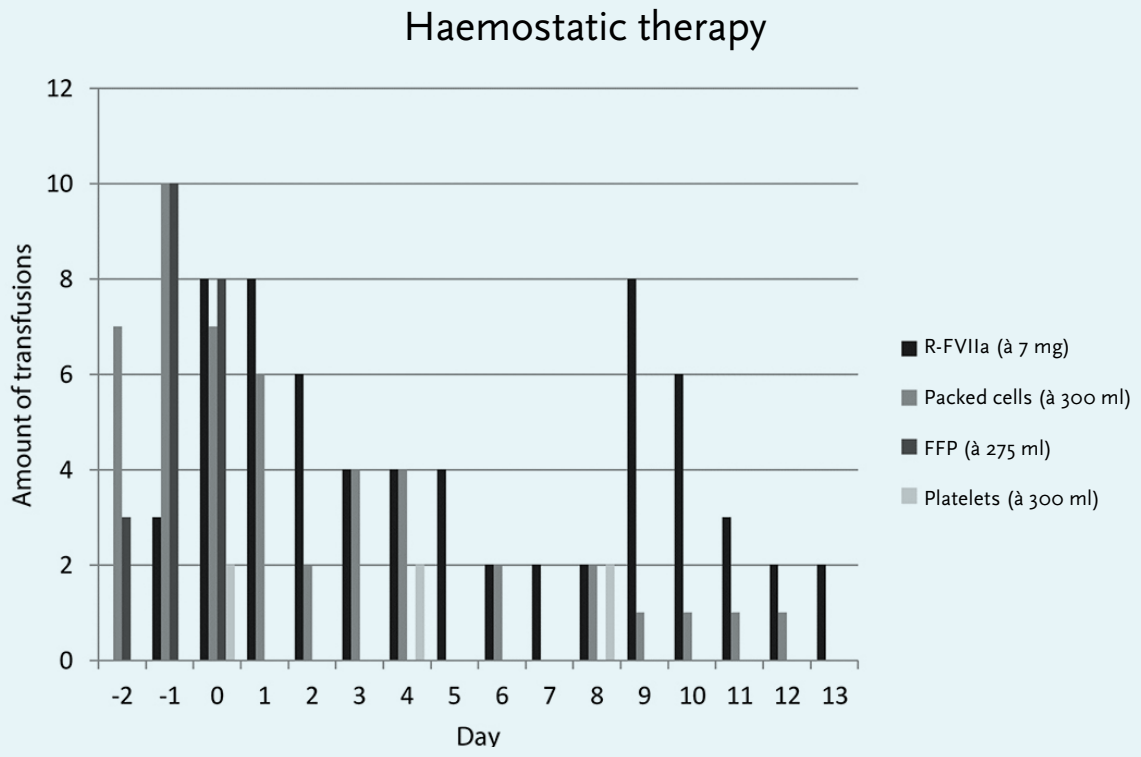
Figure 1. *CT scanning of the abdomen in the portal-venous phase showing a large haematoma (white oval) in the mesenteric fat expanding to the left upper quadrant of the abdomen. A contrast extravasation is detected on the top of the pancreaticojejunostomy suggesting active haemorrhage (white arrow)*



DISCUSSION

Here we report a patient with delayed bleeding due to acquired haemophilia A, which is a rare auto-immune

Figure 2. *Transfusion of blood products and coagulation factors after admission to the ICU. The bar chart illustrates the amount of transfusion of blood products and R-FVIIa (y-axis) after admission to the ICU (day -2; x-axis) and after start of immunosuppressive therapy (day 0; x-axis)*



FFP = fresh frozen plasma; R-FVIIa = recombinant factor VIIa

Table 1. *Laboratory results at day 0 (start of immunosuppressive therapy)*

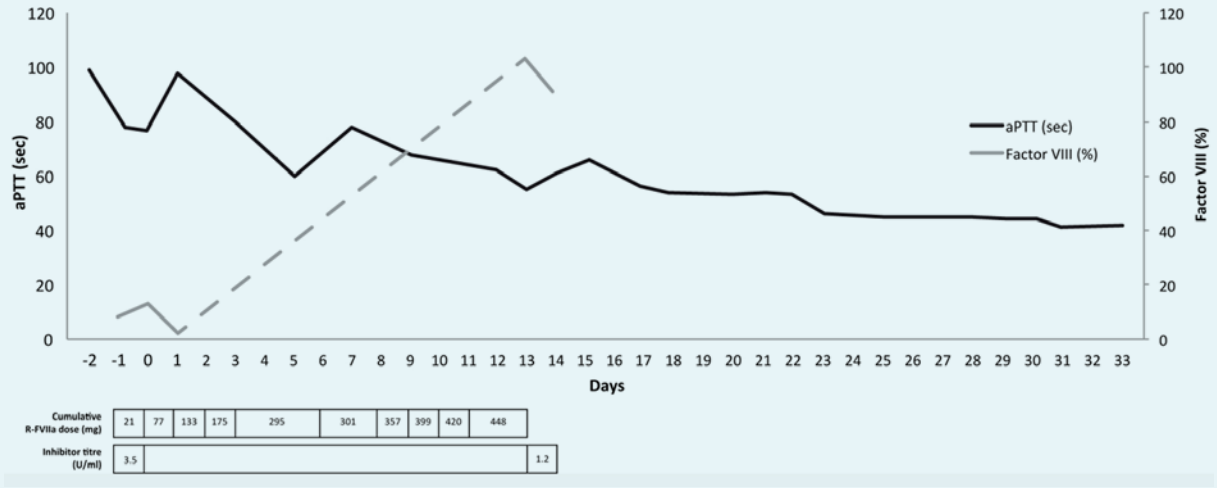
		Day 0	Reference values
Haemoglobin	mmol/l	5.5	8.5-11.0
Haematocrit	l/l	0.25	0.40-0.50
Leukocytes	10^9/l	22	4.0-10.0
Thrombocytes	10^9/l	181	150-400
aPTT	Sec	74	25.0-35.0
PT	Sec	18.4	12.5-14.5
INR		1.4	< 1.2
Fibrinogen	g/l	3.7	2.0-4.0
Factor V	%	105	70-130
Factor VII	%	24	80-120
Factor VIII	%	8	50-150
Inhibitor FVII	U/ml	<0.5	< 1.0
Inhibitor FVIII	U/ml	3.5	< 1.0

aPTT = activated partial thromboplastin time; PT = prothrombin time; INR = international normalised ratio.

disorder. In most cases the cause is idiopathic; however, it may occur in association with malignancies and some perioperative drugs (e.g. antibiotics). Although malignancy was highly suspected to be the causative factor in our patient, the aPTT was not prolonged at the time of the first surgical intervention, which makes this cause most unlikely. An isolated prolonged aPTT has a limited differential diagnosis, and after excluding the use of heparin, the primary initial diagnostic test is a mixing test (adding normal plasma in a ratio of 1:1). Persistent prolongation of the aPTT indicates the presence of an inhibitor and the Bethesda assay should quantify the antibody titre. The disease is frequently diagnosed with considerable delay despite abnormal coagulation tests (median time to diagnosis 0-8 days).[1] This suggests that many patients with severe bleeding and acquired haemophilia remain undiagnosed for a worrying length of time.

Treatment of acquired haemophilia can be divided into management of haemostasis and elimination of the inhibitor with immunosuppressive therapy. Although some patients (approximately 30%)[2,3] do not require haemostatic treatment, in the majority of the patients with acquired haemophilia bleeding is an emergency. To

Figure 3. *aPTT and factor VIII activity after admission to the ICU (day -2) and the course after start of immunosuppressive therapy (day 0). Below the x-axis the cumulative dose of R-FVIIa (mg) and the inhibitor titre (U/ml) are illustrated*



aPTT = activated partial thromboplastin time; R-FVIIa = recombinant factor VIIa

control haemostasis in these patients, two strategies can be used. First using bypassing agents (R-FVIIa, factor eight inhibitor bypassing activity (FEIBA)), and second by increasing factor VIII levels (factor VIII concentrate and desmopressin). Bypassing agents, which act by directly restoring the platelet surface factor X activation, which is deficient in the absence of factor VIIIa, are recommended as first-line therapy because of their rapid action and high level of effectiveness.[3,4] In patients with low factor VIII inhibitor titres on the other hand, high doses of factor VIII can be considered. However, as in our case, factor VIII administration may be ineffective, and to manage the severe haemorrhage, repeated administration of R-FVIIa should be initiated rapidly. The usual dose of R-FVIIa for treatment of acute bleeding is 90 to 120 µg/kg at two- to three-hour intervals until haemostasis is achieved. FEIBA is also an effective bypassing agent and comparable results to R-FVIIa are achieved at a dose of 75 IE/kg every 8-12 hours.[5] Further dosing and lengthening of R-FVIIa and FEIBA should be based upon the patient's clinical circumstances and expert opinion. In the treatment of our patient, the haemophilia treatment centre, the Van Creveld Clinic, was consulted.

The guidelines recommend immunosuppression as soon as the diagnosis has been confirmed.[4] First-line immunosuppression is usually composed of steroids alone or steroids plus cytotoxic agents, although rituximab is increasingly being used either alone or in combination with other agents.[6-8] Steroids with cyclophosphamide resulted in more stable complete remission (70%), compared with steroids alone (48%) or rituximab-based regimens (59%).[9] The median time to complete remission was approximately five weeks for steroids with or without cyclophosphamide. Rituximab-based regimens are required for approximately twice as long, which exposes patients to a longer period of increased risk of bleeding. A literature review of 71 patients treated with rituximab and a variety of immunosuppressive agents found a response rate of more than 90%, but this study was prone to positive publication bias.[10] Therefore, the current guidelines suggest that rituximab should only be used as a second-line agent in combination with steroids.[10,11] Other options for the treatment of acquired haemophilia A are administration of intravenous immune globulin or the use of extracorporeal plasmapheresis. However, given the excellent results obtained with prednisone and cyclophosphamide, these treatments are not suggested as first line.

In consultation with the haemophilia treatment centre of the University Medical Center Utrecht, the Van Creveld Clinic, we treated our patient with steroids (1 mg/kg) in combination with cyclophosphamide (100 mg), which is the most commonly used regimen in the Netherlands. With this regimen, a starting biochemical and clinical response is expected approximately two weeks after initiating immunosuppressive therapy. The duration of treatment should be based on individual patient characteristics and biochemical responses. The primary goal of treatment should be monitored; however, since inhibitor titres drop very slowly following successful treatment, it is advisable to check the inhibitor titre every two to four weeks once immunosuppressive therapy

has been started. Accurate monitoring of patients with acquired haemophilia A is necessary to detect an early relapse. The relapse rate after a first complete remission has been estimated at approximately 20%; 70% of such relapsing patients achieve a second complete remission.[1]

CONCLUSION

This case describes a patient undergoing a surgical procedure complicated by severe intra-abdominal haemorrhage due to undiagnosed acquired haemophilia A. The finding of an isolated prolonged aPTT together with the presence of ongoing haemorrhage should trigger rapid diagnostic investigations for acquired haemophilia. The start of appropriate haemostatic treatment is crucial since delayed treatment may increase mortality. Similar to the observations in the registry data, our case emphasises that patience is necessary to achieve eradication of the auto-antibodies in order to control the risk of bleeding.

DISCLOSURES

The authors declare no conflicts of interest. No funding or financial support was received.

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PHOTO QUIZ

A patient with flank pain and haematuria after allogeneic stem cell transplantation

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CASE REPORT

A 57-year-old male patient was seen at our emergency department with flank pain and haematuria that had been present for three days. Nine months earlier he had received an allogeneic stem cell transplantation (alloSCT) for acute myeloid leukaemia. This alloSCT was complicated by a cytomegalovirus reactivation and a probable pulmonary invasive aspergillosis treated with valganciclovir and voriconazole, respectively. Five months after alloSCT he developed extensive chronic graft-versus-host disease necessitating treatment with prednisolone and cyclosporine.

Figure 1. *Coronal image of the abdomen showing two hypodense lesions in the left kidney*

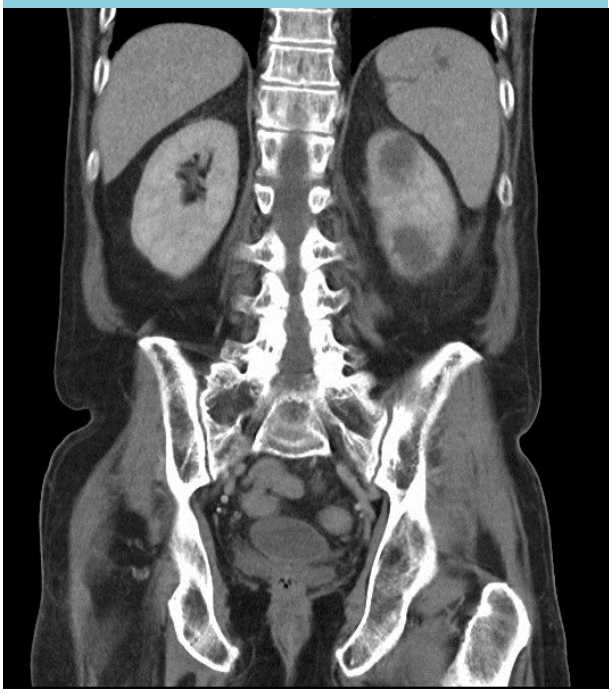
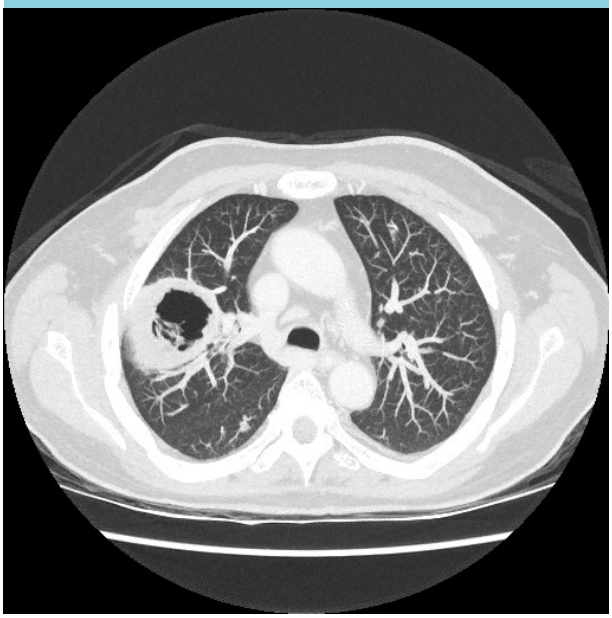


Figure 2. *Transverse section of the thorax with a cavitating lesion in the right upper lung*



On the day of admission his medication consisted of prednisolone (40 mg once daily), cyclosporine (200 mg twice daily), valaciclovir (500 mg twice daily, prophylaxis for herpes virus reactivation), trimethoprim-sulphamethoxazole (480 mg once daily, prophylaxis for *Pneumocystis jiroveci* pneumonia) and insulin because of steroid-induced diabetes mellitus.

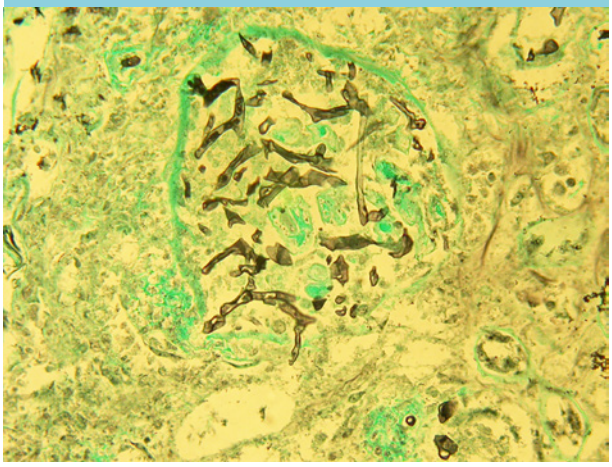
Physical examination revealed prominent left flank pain on percussion and palpation, but was otherwise unremarkable. The patient was afebrile and exhibited no pulmonary signs or symptoms. Laboratory data were as follows: leukocyte count 5.2 (normal range 4.0-11.0 x 10^9/l) with a normal neutrophil count, haemoglobin 6.6

(normal range 8.4-10.8 mmol/l), platelet count 132,000 (normal range 150-400 x 10^9/l), serum creatinine 70 (normal range 60-110 µmol/l), serum glucose 8.0 mmol/l and C-reactive protein 204 (normal range < 10 mg/l). Urine analysis showed erythrocytosis and leukocytosis, without dysmorphic erythrocytes or red blood cell casts and the nitrate test was negative.

An ultrasound was performed to evaluate the left kidney and surrounding organs and revealed a mass in the left kidney raising the suspicion of a renal cell carcinoma or lymphoproliferative disease. Therefore, a contrast-enhanced computed tomography scan of the thorax and abdomen was performed which showed two masses in the left kidney and a cavitating lesion in the right upper lung (*figure 1 and 2*).

A biopsy of the kidney (*figure 3*) and bronchoalveolar lavage were performed.

Figure 3. *Kidney biopsy shows extensive proliferation of fungal hyphae*



WHAT IS YOUR DIAGNOSIS?

See page 189 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 187)

A PATIENT WITH FLANK PAIN AND HAEMATURIA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

DIAGNOSIS

Direct microscopy of bronchoalveolar lavage (BAL) showed leukocytes, but no fungal elements. However, fungal culture was positive, and macroscopic and microscopic morphology was consistent with *Rhizopus microsporus*. The galactomannan index in the BAL was increased (3.5, cut-off value ≥ 0.5) suggesting an *Aspergillus* co-infection. No other microorganisms were found. The minimum inhibitory concentration (MIC) using the EUCAST reference method was performed, and showed a MIC of 0.5 mg/l for both amphotericin B and posaconazole.

A kidney biopsy was performed and histological examination showed focal necrosis with neutrophil accumulation and extensive proliferation of fungal hyphae, morphologically consistent with mucormycosis. Fungal cultures remained negative, and a species-specific mucormycetes polymerase chain reaction (PCR) detected *R. microsporus*, while the aspergillus species-specific PCR remained negative. A diagnosis of probable pulmonary aspergillosis and proven disseminated mucormycosis was made and treatment with liposomal amphotericin B (5 mg/kg/day) was started. Because the graft-versus-host-disease was not very active, cyclosporine was discontinued and the daily dose of prednisolone was decreased to 30 mg and thereafter gradually tapered by 5 mg per month and finally stopped.

During admission, the clinical situation deteriorated with the development of haemoptysis and anaemia. Follow-up CT scan showed progression of the pulmonary and renal lesion.

Unfortunately surgical removal of the fungal lesions was not possible and because there was evidence of fungal disease progression despite intravenous antifungal therapy and reduction of immunosuppression, it was decided to discharge the patient for outpatient palliative care. Treatment with liposomal amphotericin B, which the patient had received for approximately one month, was also discontinued. However, above expectations the patient recuperated and follow-up X-ray showed regression of the pulmonary lesion. Ultrasound of the right kidney three months after presentation showed complete regression. Posaconazole oral suspension was started (200 mg 4 times a day) and a trough level of 0.8 mg/l was achieved (target value > 0.7mg/l).

Eleven months after presentation and continued posaconazole therapy, our patient is in a stable condition. The ability to reduce the immunosuppressive therapy was believed to be the main determinant for this favourable outcome. Probably, the initial radiological deterioration which had been attributed to progression of the fungal infection was in fact a result of immune reconstitution after tapering of immunosuppressants, which caused a temporary aggravation of signs and symptoms.

Invasive mould infections are a common complication in patients after alloSCT, with an annual incidence of approximately 8.8%. The most common causative mould is *Aspergillus fumigatus*. Mucormycosis is less common with a reported incidence of approximately 3.7%.[1]

Although our patient exhibited well-recognised risk factors, i.e. acute myeloid leukaemia, alloSCT, treatment for graft-versus-host disease and diabetes mellitus, the clinical presentation was quite remarkable.

Despite the presence of a pulmonary cavity, the patient presented with flank pain. Kidney involvement is uncommon and presents in only 2% of cases, and probably results from haematogenous dissemination from the primary infection site, in this case the lung.[2]

For treatment of invasive mucormycosis, liposomal amphotericin B is the drug of first choice, and if possible, resection of infected tissue and waning of immunosuppressive drugs. Second-line or maintenance therapy may consist of posaconazole.[3,4]

Mortality depends on several factors including control over the fungus by the mentioned treatments and the ability to improve the immune status of the patient, for instance by decreasing the use of immunosuppressants. Mortality, however, remains high in patients after stem cell transplantation, with a case fatality rate of 76% in localised disease but approaching 100% in disseminated cases.[2]

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PHOTO QUIZ

Thigh mass in a 22-year-old female

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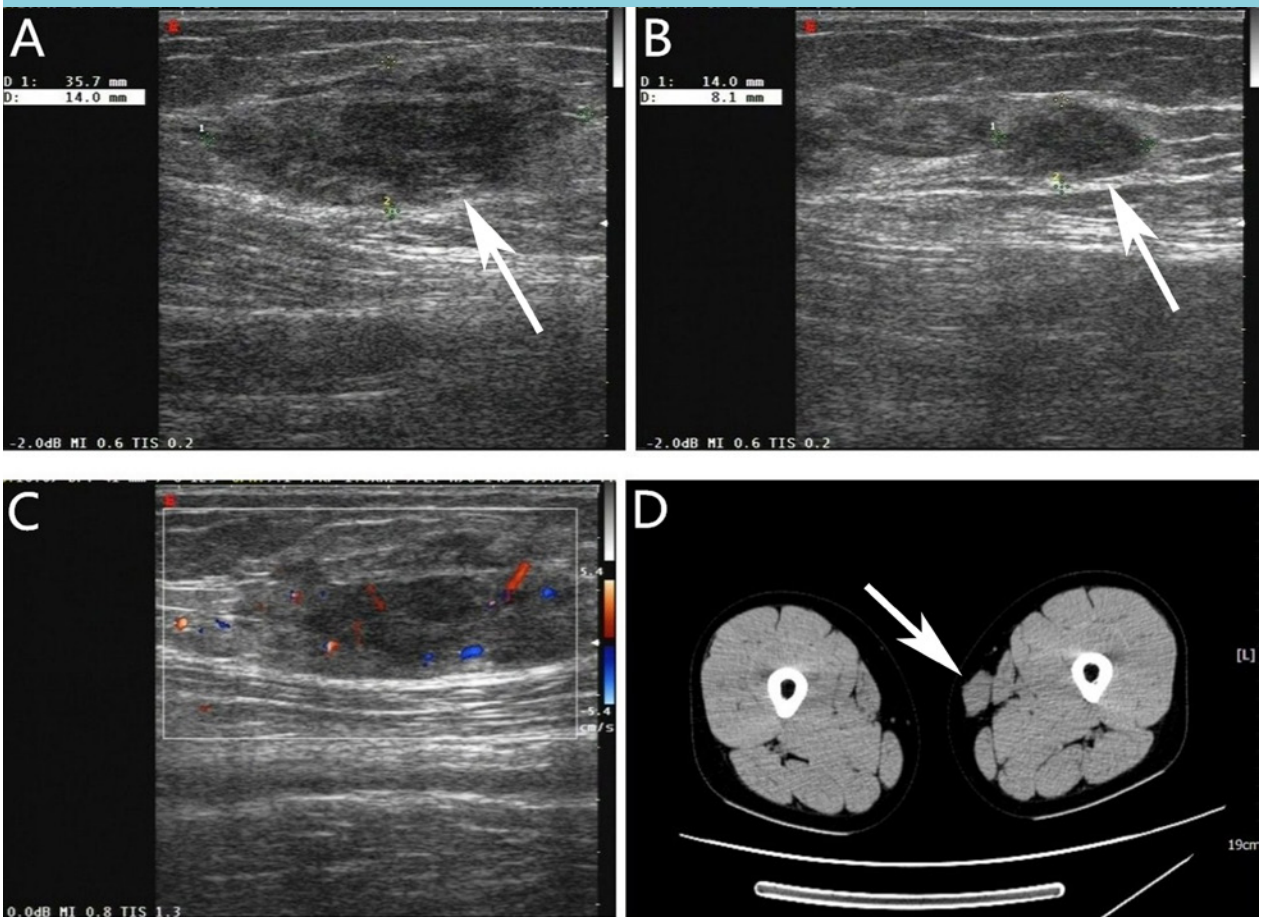
CASE REPORT

In September 2010, a 22-year-old female was admitted to our hospital with a painless and gradually growing mass in the left thigh for many months. She had no past medical history of disease and had no family history of a similar mass. The patient did not suffer from night sweats, fever or weight loss. Physical examination revealed a 3 x 2 cm painless, immobile, well-defined and firm mass in the inner left thigh. There was no lymphadenopathy in

Figure 1A, 1B and 1C. *Ultrasonic examination showed two adjacent well-circumscribed hypoechoic masses in the fat layer of the inner left thigh, which measured 3.6 x 1.4 x 2.5 cm (figure 1A) (arrow) and 1.4 x 0.8 cm (figure 1B) (arrow), respectively, with irregular shapes and inhomogenous internal echo. The blood flow signal in the masses could be explored (Blue indicator = the blood flow away from the ultrasound probe; red indicator = the blood flow to the ultrasound probe) (figure 1C)*

Figure 1D. *Computed tomography (CT) showed a well-defined soft tissue mass in the fat layer of the upper-middle inner left thigh, which measured 1.5 x 2.0 x 3.75 cm. Its density is uniform and consistent with the muscle density (arrow)*



lymph node stations or hepato-splenomegaly. Laboratory examination showed no abnormalities, including normal erythrocyte sedimentation rate (ESR), no anaemia, no haemolysis, and normal differentiation. The chest X-ray was normal. Ultrasonic examination showed two adjacent well-circumscribed hypoechoic masses in the fat layer of the inner left thigh, which respectively measured 3.6 x 1.4 x 2.5 cm (*figure 1A*) (arrow) and 1.4 x 0.8 cm (*figure 1B*) (arrow), with irregular shapes and inhomogenous internal echo. The blood flow signal in the masses could be explored (*figure 1C*). Computed tomography (CT) (*figure 1D*) showed a well-defined soft tissue mass in the fat layer of the upper-middle inner left thigh, which measured 1.5 x 2.0 x 3.75 cm. Its density is uniform and consistent with the muscle density (arrow). The patient underwent extended surgical resection of the mass on the left thigh following the initial diagnosis. Macroscopic assessment of the resected specimen identified the presence of two adjacent well-defined and firm masses, the total size of which was about 4.0 x 3.0 x 2.5 cm. Intraoperative frozen section of the specimen revealed numerous plasma cells and a few lymphocytes, and the plasma cells were at the mature phase.

WHAT IS YOUR DIAGNOSIS?

See page 192 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 190)
THIGH MASS IN A 22-YEAR-OLD FEMALE

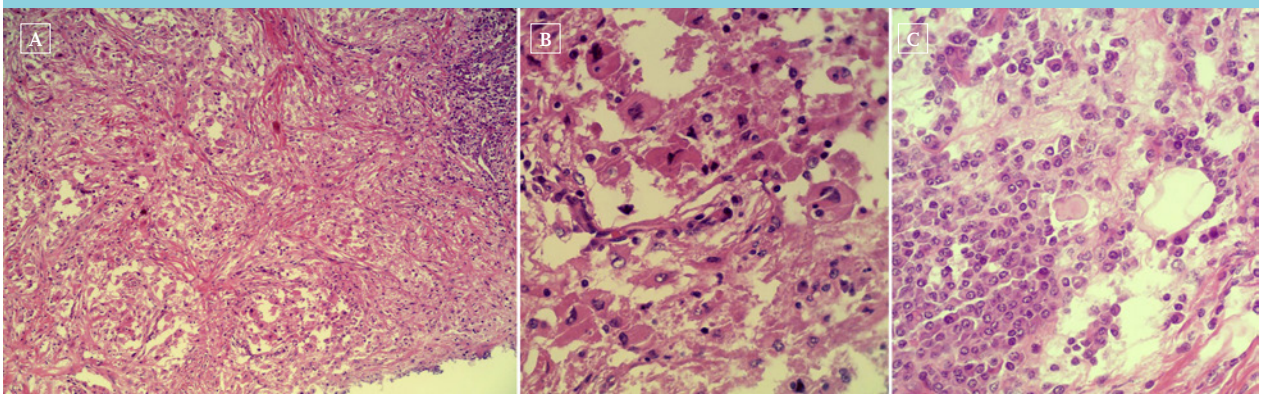
DIAGNOSIS

Final postoperative paraffin section revealed many hyperplastic histiocytes which were spindle and polygonal in shape, accompanied by infiltration of a large number of plasma cells and a small amount of lymphocytes, and interstitial fibrosis within the connective tissue (*figure 2A*). Intact plasma cells and lymphocytes were seen within the cytoplasm of the histiocytes (emperipolesis) (*figure 2B*). The infiltration of mature plasma cells and immunoglobulin-like deposits (Russell bodies and Mott cells) were also seen clearly under the microscope (*figure 2C*). A conclusive diagnosis of Rosai-Dorfman disease (RDD) of the left thigh was achieved. Immunohistochemistry staining showed that the lesions were positive for S-100, CD163, α1-antichymotrypsin, α1-antitrypsin and CD38, partly positive for CD68, λ and κ, but negative for CD1a. Immunohistochemistry staining also showed that the positive cell population of Ki-67 was < 2%. These findings also supported the diagnosis of RDD. The postoperative course was uneventful.

RDD, also known as sinus histiocytosis with massive lymphadenopathy (SHML), was first described by Rosai and Dorfman in 1969.[1] RDD is a rare and non-neoplastic histiocytic proliferative disorder of unknown cause. It is considered to be a benign and self-limited disease, but it can also have an aggressive clinical course.[2-4] RDD commonly occurs in adolescents or young adults. The typical clinical presentation of RDD is usually painless, bilateral cervical lymphadenopathy. Other clinical findings of RDD include fever, leukocytosis, anaemia, elevated ESR and hypergammaglobulinaemia.[5,6] Extranodal involvements of RDD occur in 43% of all patients with lymphadenopathy.[7] The extranodal sites include the skin, soft tissue, upper respiratory tract, orbit and bone. RDD located in the fat layer of the thigh as only an extranodal involvement without lymphadenopathy is an unusual presentation.

The definitive diagnosis of RDD relies on histopathological examination, and the immunohistochemical characteristics of the lesions contribute to determining the final diagnosis. Pathologically, RDD is characterised by a benign histiocytic proliferation with frequent lymphocytophagocytosis (emperipolesis).[6,7] RDD must be differentiated pathologically from other lesions, such as lymphoma and plasmacytoma, fibro-inflammatory lesion, Langerhans cell histiocytosis and other histiocytosis. In some cases of RDD, obvious hyperplasia of lymphocytes or plasma cells resembles the appearance of lymphoma or plasmacytoma. However, the hyperplastic lymphocytes or plasma cells are of polyclonal origin, and simultaneously express λ and κ by immunohistochemistry. Furthermore, nuclear divisions are seldom seen in the RDD. While nuclear divisions are more

Figure 2A. *Final postoperative paraffin section revealed many hyperplastic histiocytes which were spindle and polygonal in shape, accompanied by infiltration of a large number of plasma cells and a small amount of lymphocytes, and interstitial fibrosis within the connective tissue (Haematoxylin-eosin stain; original magnification x 100)*
Figure 2B. *The patchy hyperplasia of histiocytes. Besides, intact plasma cells and lymphocytes were seen within the cytoplasm of the histiocytes (emperipolesis) (Haematoxylin-eosin stain; original magnification x 400)*
Figure 2C. *The infiltration of mature plasma cells and immunoglobulin-like deposits (Russell bodies and Mott cells) (Haematoxylin-eosin stain; original magnification x 400)*



commonly seen in the lymphoma or plasmacytoma, and their lymphocytes or plasma cells are of monoclonal origin. Thus, they can be ruled out. RDD may mimic the appearance of a fibro-inflammatory lesion. But this disease can be quickly ruled out because the histiocytes of a fibro-inflammatory lesion do not show emperipolesis and are negative for S-100. Langerhans cells are generally smaller than RDD cells. In Langerhans cell histiocytosis, the reniform nuclei with an obvious nuclear groove are usually observed (unlike the round nuclei of RDD cells), and emperipolesis is very rarely observed. Furthermore, Langerhans cells ultrastructurally contain characteristic Birbeck's granules and are positive for CD1a (unlike RDD cells which are negative for CD1a). The treatment of RDD included surgical resection with or without chemotherapy, radiotherapy or corticosteroids. Our patient only underwent extended surgical resection of a mass in her left thigh without further treatment. She was followed up for four years and three months after surgery with a good prognosis.

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LETTER

PEEP in ICU patients without ARDS in the Netherlands: not a closed case

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Dear editor,

We would like to thank Schultz and colleagues for their interest in our paper and we welcome their considerations concerning our post-hoc analysis comparing Dutch with European ICU ventilation practices.[1]

In answer to their questions and concerns: the low incidence of reintubations after unplanned extubations in the Dutch cohort would indeed seem to suggest delayed planned extubations. Unfortunately, our database does not provide sufficiently detailed data to further disprove or support this.

We hypothesised that Dutch ICU patients would have received higher PEEP levels because of the works and teachings of Burkhard Lachmann, which are well known amongst Dutch intensivists. Lachmann suggested that a certain level of PEEP is needed to avoid recurring alveolar collapse with accompanying shear stress.[2] In our paper, we did not, however, recommend specific PEEP levels.

Furthermore, it is uncertain to what extent the data from the publication by Hemmes *et al.*, a randomised controlled trial in which patients were studied who underwent open abdominal surgery and mechanical ventilation during general anaesthesia, can be extrapolated to ICU patients.[3] Analysing our data, a median duration of mechanical ventilation of three days is found in both cohorts, but with a statistically significantly smaller spread in patients in Dutch ICUs (3 (2-6) vs. 3 (2-8), $p < 0.01$). In this population the association between higher levels of PEEP and longer duration of mechanical ventilation does not seem to be present.

A tendency to extubate patients at a PEEP level of 5 cm H_2O could not be found in Dutch ICU patients from our database. Median PEEP at extubation was 8 cm H_2O with an interquartile range of 5-8.

Mechanical ventilation can be both lifesaving and harmful. It is up to us to find the safest and least harmful modes of ventilation for our patients.

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LETTER

Evaluation of a vancomycin dosing protocol for intensive care unit patients

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ABSTRACT

Vancomycin is a glycopeptide antibiotic that needs to be dosed to achieve target trough levels of 15-20 mg/l. Dosing can be challenging in ICU patients. To optimise therapy, in ICU-pharmacy collaboration, a dosing protocol was introduced on the ICU of the Medical Center Leeuwarden, the Netherlands. The effectiveness in obtaining timely adequate trough levels was evaluated. We retrospectively analysed data from 59 patients. Results show that pharmacy involvement and introduction of the dosing protocol resulted in early adequate trough levels ($p = 0.016$). Introduction of the protocol alone resulted in non-significant early accurate trough levels. The protocol should be used with caution in patients with a possibly unreliable estimated glomerular filtration rate. Careful protocol introduction is important.

KEYWORDS

ICU, protocol, vancomycin

INTRODUCTION

Vancomycin is a glycopeptide antibiotic that is used in the treatment of infections caused by Gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus faecium*. Vancomycin exhibits an intricate pharmacokinetic profile, showing large inter-individual differences, especially in ICU patients.[1-3] The pharmacokinetic-pharmacodynamic (PK-PD) parameter area under the concentration curve during 24 hours (AUC) divided by the minimal inhibitory concentration (AUC/MIC) may be the best pharmacodynamic parameter to predict effectiveness of vancomycin.[4,5] However, determining this parameter in a clinical setting is complicated. Alternatively, trough serum vancomycin concentration monitoring can be used to measure dosing effectiveness in an accurate and practical manner. Correct timing of actual trough measurement is essential. Current dosing guidelines propose intermittent dosing of vancomycin with target trough levels of 15-20 mg/l.[5] Dose optimisation depends on creatinine clearance and body weight.[6] Most patients achieve target trough levels with dosages of 15 mg/kg every 8-12 hours.[5] To achieve target trough levels in critically ill patients, a loading dose of 25-30 mg/kg body weight with a maximal infusion rate of 10 mg/min can be considered.[5] Maintenance doses should be adapted to kidney function and further dose adjustments can be made based on therapeutic drug monitoring (TDM).[7] Monitoring is advised at steady state, after the fourth dose.[5] Vancomycin is associated with adverse effects, such as nephrotoxicity and ototoxicity.[7-9] ICU patients have a higher risk for development of nephrotoxicity than non-ICU patients at a lower trough concentration threshold.[10] It has been shown that early achievement of therapeutic levels in critically ill patients results in survival benefit.[11,12] Furthermore, an early response to vancomycin therapy is associated with therapy success.[13] An initial vancomycin trough level of < 15 mg/l is associated with persistent methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia (OR 4.25, 95% CI 1.51-11.96).[12] Therefore, to increase the chance of therapy success, obtaining proper trough levels in the early course of the disease is essential. The ICU and the Clinical Pharmacy Department of the Medical Centre Leeuwarden (MCL) collaborated in composing a simple vancomycin-dosing protocol for ICU patients to optimise treatment. The protocol consists of a weight-based loading dose and a maintenance dose based on the estimated glomerular filtration rate (eGFR). Guidance on therapeutic drug monitoring (TDM) is provided. Dosing recommendations, based on TDM, are given by the attending pharmacist. The goal of this

protocol is to reach rapid therapeutic trough levels of vancomycin. This study evaluates the efficacy of the dosing protocol in obtaining adequate vancomycin levels (> 15 mg/l) within 48 hours of therapy onset. A one-sided limit of > 15 mg/l was chosen since short-term trough levels of > 15 mg/l are not considered toxic and initial therapeutic failure (levels < 15 mg/l) are deemed more harmful. We also determined the time required to reach therapeutic levels beyond the initial time frame of 48 hours.

METHODS

Study population

A retrospective study was conducted at the ICU of the Medical Centre Leeuwarden. The ICU unit consists of 22 ICU beds. A search in the ICU Metavision® database was performed for patients receiving vancomycin therapy for at least 48 hours between November 2010 and May 2013. During this period three distinct study phases were identifiable. During the first phase, from November 2010 until November 2011 (group A), the pharmacy was not structurally involved in medication surveillance (e.g. checking the need for various types of TDM). During the second phase from November 2011 until September 2012 (group B), pharmacists were involved daily in medication surveillance. During the third phase from September 2012 until May 2013 (group C), the vancomycin dosing protocol was implemented next to daily medication surveillance. Patients with continuous infusion, single prophylactic doses, trough drawing > 1 hour before next dose and no known trough level were excluded. Trough levels and the time blood was drawn were obtained from the local laboratory Glimms® database. The time of vancomycin dose administration was obtained from the electronic medication administration registration. The following patient characteristics were noted: gender, age, body weight, BMI, estimated glomerular filtration rate (eGFR, ml/min/1.73 m^2, calculated with MDRD) at day 1 of vancomycin treatment, APACHE IV score and length of stay in the ICU and hospital.

Dosing protocol

Dosing advice in the consensus review by Rybak *et al.*[5] consists of a loading dose of 25-30 mg/kg for seriously ill patients and a maintenance dose of 15-20 mg/kg every 8-12 hours. We adapted this advice with the help of MW-pharm predictions (version 3.80 MediWare, Groningen, the Netherlands) to put together a practical protocol. It consisted of a loading dose of 1500 mg for patients < 70 kg and 2000 mg if > 70 kg, based on actual body weight. The next bi-daily doses were set at fixed times, but the first not earlier than eight hours after the loading dose. The bi-daily doses were 1000 mg for eGFR > 50 ml/min, 750 mg for eGFR 20-50 ml/min and 500 mg for eGFR < 20 ml/min and continuous veno-venous haemofiltration therapy (CVVH). The first TDM was performed 20-48 hours after therapy onset. The attending pharmacist made an estimated half-life based dosing recommendation, if necessary supported by MW-pharm prediction, based on initial TDM, targeted at achieving trough levels of 15-20 mg/l. Guidance on new TDM was provided.

Data analysis

In the primary analysis, groups A, B and C were compared to determine the effect of pharmacy involvement and pharmacy involvement plus dosing protocol in obtaining initial (< 48 hours) trough levels of > 15 mg/l. An intention-to-treat approach was used. The three groups were also compared to determine at which day of vancomycin therapy a known adequate trough level of > 15 mg/l was achieved.
In addition, an 'as treated' analysis was performed to account for protocol non-adherence and for patients from group B who were already treated according to the protocol.
All analyses were performed with PASW Statistics version 20.0 (SPSS Inc. Chicago, Illinois, USA).
The nonparametric Mann-Whitney U test was used to compare time of adequate trough serum levels between groups pair wise. The one-way ANOVA and nonparametric Kruskal-Wallis test were used to compare patient characteristics between groups. Categorical data were tested for statistical significance with the χ^2 or Fisher's exact test where appropriate. Results from hypothesis testing were considered statistically significant at $p < 0.05$.

RESULTS

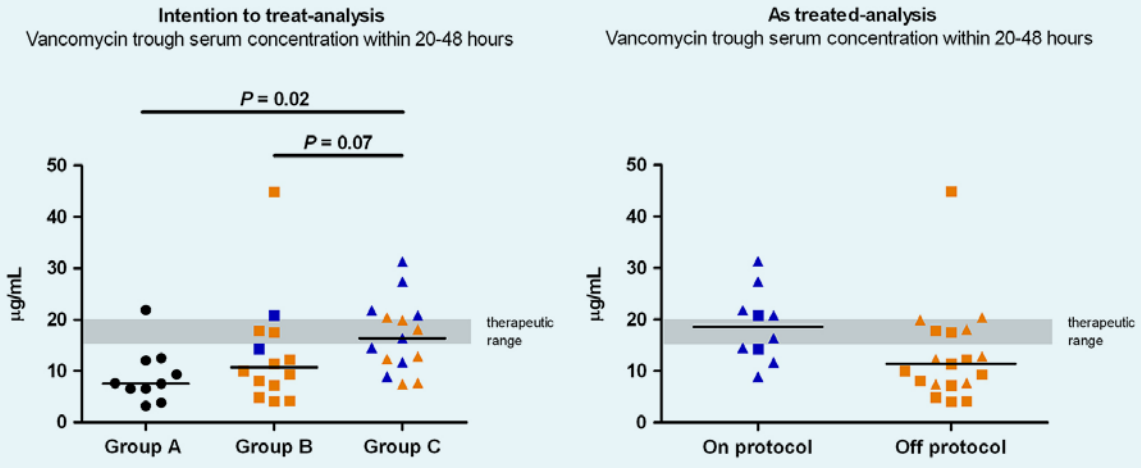
A total of 59 patients were included in the study. Patient characteristics and distribution across the three groups are shown in *table 1*.
There was a difference between the three groups in attaining adequate vancomycin trough levels within 20-48 hours, (10% in group A, 29% in B and 60% in C, p = 0.016). In groups B and C more patients achieve early adequate trough levels. Comparing pre-intervention group B (29%) with post-intervention group C (60%), a non-significant difference (p = 0.07) was found. In addition to these categorical data, *figure 1, panel A*, shows individual trough levels across groups A, B and C. When looking beyond the initial 48 hours, groups B and C both achieve adequate vancomycin trough levels earlier in comparison with group A (*figure 2*, p = 0.002). After pharmacist intervention it took an average of 1.8 days for trough levels to reach an acceptable range (< 25 mg/l). No renal replacement therapy was started because of high trough levels. Kidney function loss after vancomycin therapy in group C was assessed. One patient experienced an eGFR deterioration of more than 25% (27%).

Table 1. *Patient characteristics comparing group A (pre-surveillance), group B (pre-intervention) and group C (post-intervention)*

	Pre-surveillance n = 20	Pre-intervention n = 20	Post-intervention n = 19	p
Characteristics [median (IQR)]				
Age (years)	63 [50-75]	72 [56-77]	63 [54-71]	0.52
Body weight (kg)	84 [75-96]	84 [73-88]	73 [66-93]	0.41
BMI (kg/m²)	27 [23-29]	27 [21-30]	24 [22-28]	0.73
eGFR at day 1 (ml/min/1,73m²)	65 [50-87]	56 [45-89]	65 [46-117]	0.62
APACHE IV	74 [50-93]	77 [65-92]	85 [65-111]	0.54
Residence ICU (days)	16 [2-37]	25 [13-38]	18 [8-31]	0.76
Male (vs. female)	14 (70%)	12 (60%)	11 (55%)	0.70

BMI = body mass index; eGFR = estimated glomerular filtration rate; APACHE = Acute Physiology and Chronic Health Evaluation, calculated from ICU admission values.

Figure 1. *Graphical reproduction of individual trough levels. Panel A: comparison of groups A, B and C ('intention to treat'). Panel B: comparison of patients treated on protocol with patients off protocol ('as treated'). Horizontal lines represent median trough level values within a group. P values are for attainment of adequate vancomycin trough levels. The grey area represents the therapeutic trough range of 15-20 mg/l*



As treated

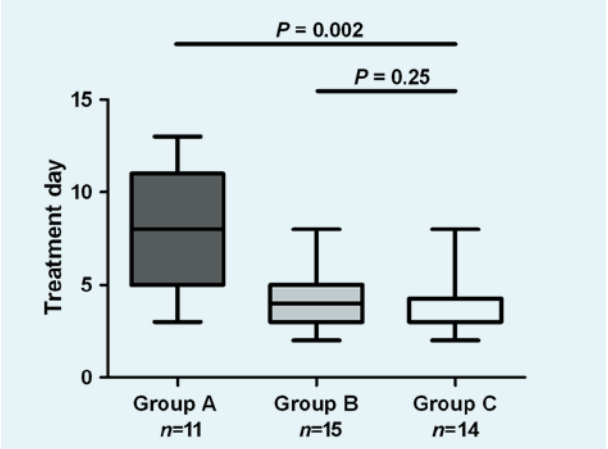
To study the actual impact of introducing the dosing protocol, treatment misclassification due to protocol non-adherence in group C (*orange triangles in figure 1, panel A*) and protocol use before official introduction in group B needed to be taken into account. Therefore, data from group B and C were pooled and compared based on protocol adherence within the first 48 hours. The results are shown in *figure 1, panel B*. Protocol adherence was defined as administration of the appropriate loading dose and maintenance dose within the first 48 hours, based on body weight and eGFR, respectively. There was a trend towards more timely trough levels in the on-protocol group (60%), compared with the off-protocol group (36%). This difference, however, is not statistically significant (p = 0.24) probably due to low patient numbers.

DISCUSSION

This study shows that pharmacist involvement and introduction of a dosing protocol at the ICU improves achievement of timely adequate vancomycin trough levels. Both the number of patients that reach timely therapeutic trough serum levels and the onset of therapeutic levels improved. ICU and pharmacy collaboration is known to optimise care.[14]

The as-treated analysis (eight patients in group C plus two patients in group B) indicated that the actual use of the treatment protocol resulted in, although not statistically significant, more timely adequate trough levels compared with manual initial dosing. Similar loading dose protocols are known to produce timely adequate trough levels as well.[15] The moderate total protocol adherence could have multiple causes. The results reported in this study were obtained just after introduction of the protocol. It is possible that it took time for each intensivist to get familiar with the protocol. Second, physicians might have refrained from using the protocol because of a perceived potential of initial toxic trough levels. The most common deviation seen was a loading dose below the one prescribed in the protocol. This problem was further reflected in the results as seen in *figure 1, panel B*. Especially the two highest trough levels of 27 and 31 mg/l might have caused restraint because of the risk of nephrotoxicity. However, careful analysis of the characteristics of these two patients showed that they were admitted to the hospital for over three weeks, including several days in the ICU, before being treated with vancomycin. The eGFR, which is based on serum creatinine, could have been overestimated in these cases, resulting in too high vancomycin dosing and high trough levels. Therefore, caution seems warranted while using the protocol in patients who may have an unreliable eGFR. A limitation of this study is that an uncorrected eGFR in ml/min/1.73 m^2 was used to determine the maintenance dose. However, even if corrected for body surface area, use of all formula based estimates has limitations, especially in ICU patients. At the time of protocol introduction, no better alternative for kidney function assessment was possible. The general limitation of this study is the retrospective design with only a limited number of patients. This was further complicated by non-adherence and missing data. However, this is also the strong point of the study in that it reflects daily clinical practice well and brings to light problems in the introduction and use of a dosing protocol in clinical practice.

Figure 2. *Box and whiskers plot of day that patient reaches trough serum level of > 15 mg/l. Medians for group A, B and C are 7.7, 4.4 and 3.8, respectively. **p < 0.01 compared with group A. Cessation of vancomycin therapy before adequate trough levels results in missing values*



CONCLUSION

We observed that the introduction of our vancomycin dosing protocol and pharmacist involvement resulted in timely adequate vancomycin trough levels in patients admitted to the ICU. A careful introduction, also focusing on protocol adherence, needs attention.

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