“Take a look at this finger: what is your diagnosis?”

Bone and glucose metabolism
- Epidemiology of anal cancer
- Iron deficiency and bariatric surgery
- Aetiology of community-acquired pneumonia
- Malaria recrudescence after 2½ years
- Venomous snakes in the Netherlands
- Hypocalcaemia after pamidronate infusion
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The mission of the journal is to serve the need of the internist to practise up-to-date medicine and to keep track with important issues in health care. With this purpose we publish editorials, original articles, reviews, controversies, consensus reports, papers on speciality training and medical education, book reviews and correspondence.

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Certainty in medicine: A moving target

M. Levi

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In medical school students are often impressed, if not beleaguered, by clinical teachers with bold statements that they should never forget. ‘Do not let the sun go down over a diabetic ketoacidosis’ or ‘Giving morphine to a patient will spoil the evaluation of an acute abdomen’ are examples of frequently lectured, sometimes even apodictic, proclamations. Many of these clinical edicts, however, have turned out to be wrong with current insights. In my personal medical training, not even very long ago, we were taught that duodenal ulcers were due to ‘type A personality disorders’ (as exemplified by taxi drivers), that patients with heart failure should never be given a beta-blocker, and that patients with type 2 diabetes mellitus could not develop ketoacidosis. All this turned out to be untrue as well, not based on any empiric scientific observation, let alone understanding of underlying pathogenetic mechanisms. Nevertheless, many students, now practising physicians, remember these aphorisms and find it difficult to get rid of them. Despite all postgraduate educational efforts, state of the art lectures during medical congresses and continuous medical education programs, many doctors still believe that oedema in nephrotic syndrome is due to low albumin levels, that humans have an intrinsic and extrinsic coagulation system, or that diverticulitis or pancreatitis should always be treated with antibiotics. Obviously, we now know that all this is incorrect, as recent publications also in the Netherlands Journal of Medicine underscore.

And the list of once ‘true’ but nowadays outdated medical knowledge is endless, as biomedical knowledge and its application to medicine has been developing very rapidly over the last decades. Medicine is moving fast and practising physicians have to keep up with it. How can we do that? Indeed by attending continuous medical education sessions and participating in postgraduate activities. And by reading medical journals in our field and in general areas of medicine. One of the main objectives of the Netherlands Journal of Medicine is to provide such an information source for practising physicians in internal medicine at large and to keep our readership up-to-date on new medical knowledge and its application in day-to-day medicine. In this issue of the Netherlands Journal of Medicine surprising new knowledge on bone as a pivotal regulator of glucose homeostasis is reported as well as important new insights into the epidemiology but also (viral) aetiology of anal cancer. This new information may soon be applicable for better management of these conditions. But, in the same issue, information is reported that has already been translated to clinically applicable knowledge on diseases as common as community acquired pneumonia or (at least in the Western world) as rare as malaria.

Keeping up to date in medicine is an ongoing and never-ending battle that each physician has to deliver. Individual strategies, tailored to personal and specific needs, will be important to stay on board and will require considerable efforts but at the same time will keep our working life in medicine vibrant and exciting. The Netherlands Journal of Medicine hopes it will contribute for a small part in this endeavour.

REFERENCES


Bone as a regulator of glucose metabolism

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ABSTRACT

For a long time the only functions attributed to the skeleton were locomotion and calcium storage. Over the last decade, this view has changed. Genetic studies in mice have shown that bone metabolism is regulated by the autonomic nervous system and interacts with energy metabolism and reproduction. Osteocalcin, one of the main organic ingredients of the bone matrix, was discovered to stimulate insulin production by the pancreas, as well as energy expenditure and insulin sensitivity. Administration of recombinant osteocalcin to mice on a high fat diet decreased weight gain and insulin resistance. These unanticipated results stimulated studies on osteocalcin and glucose metabolism in humans. This review will discuss these clinical studies and their perspective for the future.

KEYWORDS

Bone metabolism, glucose metabolism, insulin, osteocalcin, review

INTRODUCTION

For a long time the only functions attributed to the skeleton were locomotion and calcium storage. Over the last decade, this view has changed. Genetic studies in mice have shown that bone metabolism is regulated by the autonomic nervous system and interacts with energy metabolism and reproduction (reviewed in references 1-5). This review will focus on the interaction between bone metabolism and glucose metabolism and will highlight animal experimental research with potential towards clinical application.

INsulin and Bone

It is well recognised that diabetes patients have an increased fracture risk. However, bone mineral density is affected differently in diabetes type 1 and 2 patients. Diabetes mellitus type 1 (DM1) patients have a lower bone mineral density whereas diabetes mellitus type 2 (DM2) patients have a higher bone mass than healthy individuals. The mechanisms for these differences are not completely understood, but one of the hypotheses is that insulin is an anabolic factor for bone. As a consequence, DM1 patients, with a lack of insulin, do not attain their peak bone mass which leads to lower bone mineral density and a higher risk of fracture while DM2 patients are hyperinsulinaemic which stimulates bone accrual. This hypothesis has been strengthened by in vitro experiments showing that osteoblasts express the insulin receptor and addition of insulin to osteoblast cultures promotes survival and collagen synthesis. Although bone mineral density is increased in DM2 patients, the quality of the bone is probably lower, possibly due to the hyperglycaemia, leading to an increase in fracture risk. Furthermore, the risk of falls promoting fracture is increased especially in DM2 patients; the reasons for this include medication use, hypoglycaemic episodes and gait instability because of neuropathy and visual impairment.

Bone and Insulin

Karsenty et al. were the first to hypothesise that bone exerts a reciprocal influence on insulin metabolism. They reasoned that the skeleton is a very large organ and its maintenance consumes vast amounts of energy, making a link between the skeleton and energy supply plausible. By screening for bone-specific genes and subsequently generating knockout mice of these genes to study the metabolic phenotypes, osteocalcin and embryonic stem
cell phosphatase (Esp) became likely candidate genes involved in energy metabolism. Osteocalcin is one of the main organic ingredients of the bone matrix and exists in an undercarboxylated and carboxylated form. Carboxylation of its glutamic acid residues increases its affinity for hydroxypatite, facilitating its engraftment in the bone matrix. Osteocalcin knockout mice, which have been studied before in the context of bone metabolism, turned out to be obese and poor breeders. Metabolically, these mice exhibited hyperglycaemia, low insulin levels, low beta cell mass, low insulin sensitivity and low energy expenditure. The phenotype of heterozygous Esp knockout mice posed a mirror image of the osteocalcin knockout mice. Esp encodes the enzyme osteotesticular protein tyrosine phosphatase (OST-PTP), Esp is expressed solely in osteoblasts and Sertoli cells and OST-PTP inactivates the insulin receptor in the osteoblast. Therefore in Esp knockout mice the insulin receptor in the osteoblast is constitutively active. Esp knockout mice had increased osteocalcin concentrations, were lean with high energy expenditure, and had increased glucose tolerance and insulin sensitivity. At the same time, the research group of Clemens reported the phenotype of the osteoblast-specific insulin receptor knockout mouse which turned out to be osteopenic with low osteocalcin serum concentrations, obese and insulin resistant.

Further investigations into the relation between osteocalcin, OST-PTP and glucose metabolism showed that insulin, upon binding to the insulin receptor on the osteoblast, promotes osteocalcin gene expression and decreases the expression of the gene osteoprotegerin (OPG). OPG normally impedes osteoclast differentiation; therefore, insulin signalling on the osteoblast stimulates bone resorption by the osteoclast. During bone resorption, osteoclasts create an acidic environment to dissolve bone matrix. Osteocalcin is released from the bone matrix and because of the low pH, the glutamic acid residues on osteocalcin become decarboxylated and the concentration of undercarboxylated osteocalcin in the circulation rises. Finally, binding of undercarboxylated osteocalcin to the receptor GPCR6a on the pancreatic beta cell stimulates insulin secretion (figure 1).

Infusion of recombinant osteocalcin into wild-type mice indeed improved glucose tolerance and increased insulin secretion. Furthermore, when infused in mice on a high fat diet, osteocalcin reduced weight gain and insulin resistance.

**CLINICAL STUDIES**

**Glucose metabolism**

Following the discovery of osteocalcin as a regulator of glucose metabolism in mice, many researchers started reporting on the association between osteocalcin levels and measures of glucose metabolism in humans. Since osteocalcin deficient mice are hyperglycaemic, it was expected that humans with lower osteocalcin levels would have higher indices of glucose metabolism, such as fasting plasma glucose, insulin and HOMA index. Several studies indeed confirmed this inverse relation in postmenopausal women, obese patients, men and older patients. In addition, a compensatory increase in osteocalcin was shown in prediabetes and lower osteocalcin predicted the development of diabetes over ten years of follow-up in men with an increased risk of diabetes. Additional studies showed the same inverse relation between osteocalcin and the metabolic syndrome, coronary atherosclerosis, fat mass and intima-media thickness and non-alcoholic fatty liver disease.

From these studies, there seems to be an association between osteocalcin and glucose or insulin metabolism, which is compatible with the mouse models. However, in all of the reported studies, total osteocalcin was measured and in the studies that measured both total and undercarboxylated osteocalcin the relation was observed for total osteocalcin only, whereas the studies in mice centred on undercarboxylated osteocalcin. To solve this inconsistency, it would be necessary to prospectively evaluate the effect of osteocalcin on glucose metabolism in an intervention study. However administration of recombinant osteocalcin to humans has not been reported yet.

**Bone metabolism**

Another approach is to investigate the effects of interventions in bone metabolism which affect osteocalcin concentrations on glucose metabolism. It was expected that a decrease in undercarboxylated osteocalcin as observed during bisphosphonate treatment would have a negative effect on glucose homeostasis. And vice versa, that treatment with parathyroid (PTH) hormone, increasing bone formation and osteocalcin concentrations, would protect against glucose metabolism derangements. But no difference in fasting glucose or the glucose/insulin ratio was observed comparing patients treated with alendronate or PTH, although the osteocalcin concentrations changed several-fold. Contrary to the hypothesis, bisphosphonate users had a dose-dependent reduced risk of diabetes compared with matched controls with a dose-response effect. In addition three large, randomised, placebo-controlled trials of alendronate (FIT trial), zoledronic acid (HORIZON-PFT) and denosumab (FREEDOM) showed no effect on fasting glucose, body weight or diabetes incidence.

**Vitamin K metabolism**

Another possible interventional approach to modulate osteocalcin concentrations comes from vitamin K
metabolism. Vitamin K is essential for the carboxylation of glutamic acid residues in several proteins, including osteocalcin. Vitamin K deficiency increases undercarboxylated osteocalcin and supplementation of vitamin K reverses this effect. Therefore, supplementation of vitamin K was expected to have a negative effect on glucose metabolism. Several randomised controlled trials demonstrated that vitamin K supplementation decreased undercarboxylated osteocalcin, but the effect on glucose metabolism varied from an increase in insulin sensitivity in younger men, no effect on glucose metabolism in women, to an increase in insulin concentrations in older men.

**Osteoid osteoma**

Finally, a recent case report on osteoid osteoma patients was considered a proof of principle of the action of osteocalcin in humans. Osteoid osteoma is a benign osteoblastic tumour shown to secrete osteocalcin. Two young male patients, who had this tumour removed, were compared with two matched patients undergoing knee surgery and with three healthy controls. Surgical resection of the tumour was followed by a decrease in serum total osteocalcin accompanied by an increase in serum glucose and insulin, representing some degree of insulin resistance in the osteoma patients but not in the two control groups. Undercarboxylated osteocalcin concentrations were not reported.

**Conclusion and Discussion**

The association of diabetes with impaired bone metabolism has been longstanding. Strong evidence from experimental studies in genetically modified mice indicates that the bone derived hormone osteocalcin interacts with glucose and insulin secretion and possibly insulin action. This led to the proposal of the
bone-pancreas endocrine axis and spurred a wealth of studies investigating the mechanism in humans. So far, many post-hoc analyses of observational studies have confirmed the inverse relation between osteocalcin and parameters of glucose and insulin metabolism. However, only a few studies measured the undercarboxylated form of osteocalcin, which is known to be the hormonally active form in mice. Furthermore, the inverse relation between osteocalcin and glucose was not observed in several interventional studies. Since observational studies do not prove causality and the interventional studies do not support the hypothesis, the question remains whether osteocalcin has the same role in the regulation of energy metabolism in mice as in humans.

One of the possible explanations for the difference in mice and humans could be a genetic difference; humans have only one osteocalcin gene whereas mice have three. The protein sequence is conserved for 60% in mice compared with humans. In humans, the promoter of the osteocalcin gene is upregulated by vitamin D whereas the mouse gene is downregulated (reviewed in reference 46). Another explanation concerns the mouse model used in these experiments; knockout mice have a total lack of osteocalcin, whereas in human physiology osteocalcin levels may vary, but will never be completely absent. This will probably make it more difficult to pick up subtle effects. On top of this, serum osteocalcin exhibits diurnal variation and is increased during growth and skeletal maturation, ageing and menopause 47,48 which could influence the associations obtained in cross-sectional research designs.

Furthermore the role of vitamin K should be considered. Since the bone-pancreas axis is controlled by osteocalcin released from the bone and decarboxylated by the acidification of osteoclasts, the vitamin K dependent carboxylation in the circulation could influence the feedback loop. In humans the percentage of undercarboxylated circulating osteocalcin is supposed to be a marker of vitamin K intake and most studies did not take into account vitamin K concentrations or intake. 47-48 This is also an important limitation of the osteocalcin infusion studies in mice, since any clinically relevant change should be compared with the changes in concentrations with vitamin K intake. In addition, the measurement of osteocalcin and its carboxylated and undercarboxylated form is still a challenge and the interpretation could bias the results. 49,50

Therefore, the question remains whether changes in osteocalcin mediate an effect on glucose metabolism or, the other way around, whether rising glucose concentrations and changing insulin concentrations in diabetes affect bone metabolism by influencing osteocalcin concentration. The evidence from the current studies is inconclusive to answer this question definitively. Finally, an enticing question is whether it is ‘just’ osteocalcin and glucose metabolism or whether there are additional bone hormones which could influence not only glucose but also other processes of energy metabolism. Notwithstanding these limitations, the unravelling of a new endocrine axis involving bone and glucose metabolism is very exciting and the prospect of novel therapeutic options for the treatment of obesity and diabetes is worth the effort.

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Veldhuis-Vlug et al. Bone as a regulator of glucose metabolism.
The increasing incidence of anal cancer: can it be explained by trends in risk groups?

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ABSTRACT

Background: Anal cancer incidence is gradually increasing. The cause of this increase is not exactly known. This systematic literature review aimed to investigate the trend in time of anal cancer incidence and to find an explanation for the supposed increase.

Methods: The TRIP database and PubMed were searched for trends in time in incidence of anal cancer in the general population, for risk factors and risk groups for anal cancer, and for incidence trends in time in these risk groups.

Results: Age-adjusted incidence rates have increased in all Western countries during the last decades, up to 2.2% per year. Infection with the oncogenic human papilloma virus is the most important aetiological factor. Besides increasing age, other risk factors have been identified: smoking, sexual practices, in particular receptive anal intercourse, and being human immunodeficiency virus (HIV) positive. The standardised incidence ratio (SIR) is significantly increased in HIV-positive men who have sex with men (MSM) (SIR 77.8), organ transplant recipients (SIR approx. 6) and women with a history of cervical cancer (SIR 6) or cervical intraepithelial neoplasia (SIR 16). Absolute numbers of HIV-positive MSM and organ transplant recipients have increased significantly in the last decades.

Conclusion: The increasing incidence of anal cancer can be partially explained by an increase in the incidence rate in and absolute number of the most important risk group: HIV-positive MSM. The increasing number of renal transplant recipients probably also contributes. Further studies should answer the question whether these risk groups would benefit from preventive screening for anal cancer.

KEYWORDS

Anal cancer, HIV, incidence, risk groups

INTRODUCTION

Anal cancer originates from or nearby the transition zone in the anal canal. The squamous cell carcinoma is the most common form of anal cancer. Other, more rare, tumours of the anal canal are adenocarcinomas, anal melanomas, anal sarcomas and anal neuroendocrine tumours. As with cervical cancer, anal squamous cell carcinoma is caused by a persistent infection with the sexually transmitted oncogenic human papillomavirus (HPV). Several studies from different countries have reported an increase in anal cancer incidence during the last 20 years. In the Netherlands, for example, the incidence of anal cancer has doubled during the last decennia. Although anal cancer remains relatively rare in the general population, it accounts for a significant burden of disease in certain risk groups. The cause of this reported increasing incidence of anal cancer is relatively unknown, but an important role is attributed to the increase in the number of immunocompromised persons. Studies have shown that human immunodeficiency virus (HIV)-positive persons and men who have sex with men (MSM) are at increased risk for anal cancer. Organ transplant recipients and women with a history of cervical cancer or cervical intraepithelial neoplasia are also known to have a greater risk for anal cancer. This literature review aimed to investigate the trend in time of anal cancer incidence in Western countries and to find an explanation for the supposed increase. We therefore explored whether there is indeed a change in incidence of anal cancer since 1970. Next, we tried to explain the grounds of this change by identifying known risk factors and risk groups of anal cancer. Finally, we focussed on changes in anal cancer incidence among these risk groups, to see whether such changes, if present, could explain the overall increase in anal cancer incidence. The findings of this literature review might be of help in identifying risk groups who could benefit from adequate prevention measures, including screening for anal cancer by high-resolution anoscopy.
Two comprehensive literature searches were performed. For the first search, focussing on trends in the incidence of anal cancer, the TRIP database was searched in February 2012, using the term ‘anal cancer’ and the combination ‘anal cancer’ and ‘incidence’. The Medline/PubMed database was searched for data from 1970 onwards on the incidence of anal cancer. This search was restricted to the English and Dutch language. The exact search was:

```plaintext
("Anus Neoplasms"[Mesh]) OR (anal cancer[tiab]) OR (anal carcinoma[tiab]) OR (anal intraepithelial neoplasia[tiab])
AND ("Incidence"[Mesh]) OR (incidence[tiab])
```

Limits: English, Dutch, Publication Date from 1970/01/01
From both the TRIP and PubMed searches, relevant studies on trends in time in the incidence of anal cancer in the general population were identified, as well as specific studies on the trends in time among the risk groups HIV-positive persons, MSM, organ transplant recipients and women with a history of cervical cancer or cervical intraepithelial neoplasia.

For the second search, focussing on risk factors and risk groups, the TRIP database was searched in February 2012, using the terms ‘anal cancer’ and ‘etiology’, ‘anal cancer’ and ‘risk factors’ and ‘anal cancer’ and ‘risk group’. The Medline/PubMed database was searched for reviews (from 2007 through June 2012) on risk factors and risk groups for anal cancer. This search was restricted to the English and Dutch language. Initially, this search included original studies from 1970 onwards. This yielded too many articles on risk factors and risk groups. Therefore, we restricted the search to reviews from 2007 onwards. We assumed that data published before this date will have been covered by these recent reviews.

The exact search was:

```plaintext
("Anus Neoplasms"[Mesh]) OR (anal cancer[tiab])
OR (anal carcinoma[tiab]) OR (anal intraepithelial neoplasia[tiab])
AND ("Epidemiologic Factors"[Mesh]) OR (Etiology[Subheading]) OR (Etiology[tiab]) OR (Risk factor[tiab]) OR (Risk group[tiab])
```

Filters: Publication date from 2007/01/01; Review; English; Dutch

Reference lists of the retrieved articles were reviewed to identify the original studies on risk factors and risk groups. These studies were also retrieved.

Studies on the incidence of anal intraepithelial neoplasia (AIN) and HPV infections only, studies that did not report population-based incidence rates, and studies that did not distinguish between colorectal cancer and anal cancer were excluded. Studies on incidence rates of anal cancer from other than Western countries (i.e. Northern America, Western Europe and Australia) were also excluded, since most of these countries lack nationwide databases that go back far enough in time to see trends.

Additional data on the incidence of anal cancer in the Netherlands were obtained from the Dutch Cancer Registry (NKR) database (http://cijfersoverkanker.nl/). Incidence data of anal cancer in the United States were obtained from the Surveillance, Epidemiology and End Results (SEER) Program database of the US National Cancer Institute (http://seer.cancer.gov/). We did not find other easily accessible national cancer registries. Additional data on the proportion of HIV-infected MSM in the total HIV-positive population in follow-up in the Netherlands were obtained from the 2007 and 2011 Monitoring Reports of the Dutch HIV Monitoring Foundation (http://www.hiv-monitoring.nl/).

**Interpretation of data**

The International Classification of Diseases for Oncology, Third Revision, (ICD-O-3) codes C21.0-C21.8, which corresponds with International Classification of Diseases for Oncology, Tenth Edition, (ICD-10) codes C21.0-C21.8, is most commonly used to classify anal tumours. All studies reported used this or similar histological and topographical classifications of anal cancer, unless otherwise specified.

Age adjustment of anal cancer rates is necessary, since cancer is more prevalent among the elderly (figure 1).

**Figure 1. Age-specific SEER incidence rates by sex: anus, anal canal and anorectum, all ages, all races 1992-2009; derived from reference 13**

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Cancer sites include invasive cases only unless otherwise noted.
Incidence source: SEER 13 (San Francisco, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Seattle, Utah, Atlanta, San Jose-Monterey, Los Angeles, Alaska Native Registry and Rural Georgia).
Rates are per 100,000.
Datapoints were not shown for rates that were based on less than 16 cases.

Van der Zee et al. Trends in anal cancer incidence.
Countries or regions with an increasing number of elderly people would therefore get erroneously increasing incidence rates without age adjustment. All studies used age adjustment, unless otherwise specified. Differences in incidence rates over time were considered significant at p value <0.05.12,13

**RESULTS**

**Incidence of anal cancer and trends in time**

The first search in the TRIP database, using the term ‘anal cancer’, yielded 291 articles of the level of secondary evidence, from which three studies seemed relevant after selection of the title and abstract, and they were used. The other search in the TRIP database, combining the terms ‘anal cancer’ and ‘incidence’, yielded 129 articles, none of which seemed relevant. The first Medline/PubMed search, focussing on the incidence of anal cancer, identified 453 articles, from which 104 articles seemed relevant by title and abstract. Eventually 12 articles were used for the general incidence rates of anal cancer (figure 2). One additional article was found by checking the reference lists of retrieved articles.

We found that recent age-adjusted incidence rates of anal cancer differ between Western countries, with rates ranging from 0.7 per 100,000/year in the United Kingdom,7 0.83 in the Netherlands,14 1.35 in Australia10 to 1.7 per 100,000/year in the United States.13 In most countries, incidence rates are higher for women. Incidence

**Figure 2. Results of systematic literature search on general incidence rates of anal cancer and incidence rates among specific risk groups for anal cancer**
rates have increased in all Western countries during the last decades (table 1). Except for Canada, incidence rates of anal cancer increased in both sexes for the studied countries.

Data for the Netherlands
Incidence data of anal cancer in the Netherlands were retrieved from the Dutch Cancer Registry (NKR) database. It includes all cancer diagnoses of the anal canal and anus and is age adjusted using the European standard population.

The age-adjusted incidence rate of anal cancer in the Netherlands in 1989 for both sexes combined was 0.45 per 100,000 inhabitants and the gender-specific rate was higher for men than for women. The incidence increased significantly (84%) between 1989 and 2010, with the increase being more prominent in women than in men (102% versus 67%) (figure 3). In 2010 the overall incidence was 0.83 per 100,000, and it is slightly higher for women (0.85 per 100,000) than for men (0.82 per 100,000). In 2011, 40 persons died from anal cancer in the Netherlands.

Aetiology, risk factors and risk groups
The searches in the TRIP database, using the terms ‘anal cancer’ and ‘etiology’, ‘anal cancer’ and ‘risk factors’ and ‘anal cancer’ and ‘risk group’ yielded 60, 137 and 178 articles of the level of secondary evidence, respectively. None of these articles seemed relevant. The Medline/PubMed search, focussing on risk factors and risk groups, identified 95 reviews, from which 37 reviews seemed relevant by title and abstract. Eventually 22 reviews were used (figure 4).

<table>
<thead>
<tr>
<th>Table 1. Age-adjusted incidence rates of anal cancer and changes over time in anal cancer incidence by country, gender and period</th>
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*No confidence interval available; †p < 0.05; ‡p < 0.001.
Van der Zee et al. Trends in anal cancer incidence.

For a long time, anal cancer was thought to develop as a result of chronic irritation from haemorrhoids, anal fissures, fistulae and inflammatory bowel disease. More recent studies have rejected these ideas and identified certain aetiological factors, risk factors and risk groups.1,4

Smoking
Several studies have confirmed cigarette smoking to be a risk factor for the development of anal cancer. A study in 1992 estimated the odds ratios (OR) for anal cancer among smokers versus non-smokers at 3.0 (95% CI 1.9-5.0) for women and 5.0 (95% CI 1.6-16.1) for men, with the risk decreasing after cessation of smoking.16 More recently, a study confirmed the risk of smoking for anal cancer for men (adjusted OR=3.9; 95% CI 1.9-8.0) and women (adjusted OR=3.8; 95% CI 2.3-6.2), without variation in age.17 A population-based case-control study from Denmark and Sweden found that the risk increased linearly by 6.7% per pack-year.18 An earlier study already found a correlation between pack-years of smoking and anal cancer (RR=1.9 for 20 pack-years, p value <0.001; RR=5.2 for 50 pack-years, p value <0.001).19-21

HPV infection
Infection with human papillomavirus is the most prevalent sexually transmitted disease with approximately 75% of all sexually active people infected during lifetime.22 Normally the virus is rapidly cleared and genital warts (caused by the non-oncogenic HPV types 6 and 11) will only develop in 1% of infected patients.1,23 Infection with oncogenic HPV types (HPV-16 and HPV-18) is the most important aetiological factor for anal cancer.2,24 A population-based case-control study in Denmark and Sweden tested 386 patients with anal cancer and found oncogenic HPV types in 90% of women and 64% of men.25 A similar study in the United States detected oncogenic HPV in 87.9% of 262 anal cancers. No differences were reported between sexes.17 Of the more than 120 subtypes of HPV, HPV-16 was shown to be the most frequently (70%) detected in patients with anal cancer.1,17,18,26 A recent systematic review, combining worldwide data, found a prevalence of 65.6% of HPV-16 and 5.1% of HPV-18 in anal cancer.27

Similar to cervical cancer, anal cancer seems to be preceded by a premalignant lesion, called anal intraepithelial neoplasia (AIN), which is also associated with HPV infection.1,28 Anal condylomata are associated with anal cancer as well.2,24 Because genital warts are caused by HPV-6 or HPV-11, which are not oncogenic, the association of genital warts with anal cancer is more likely to be a marker for high-risk sexual behaviour resulting in co-infection with oncogenic HPV subtypes.19,30

Sexual practices
Several studies have investigated the relationship between sexual practices and the risk for anal cancer. Early population-based case-control studies in 1987 and 1989 have shown that men who were never married, who have not been exclusively heterosexual and men who have practised receptive anal intercourse had higher risks for anal cancer.19,29 Another population-based case-control study in 2004 confirmed these findings by showing that the risk of anal cancer was higher in men who were not exclusively...
heterosexual (OR=17.3; 95% CI 8.2-37.6). Among these men, practising receptive anal intercourse was independently strongly related to the risk of anal cancer (OR=6.8; 95% CI 1.4-33.8). Men who have had more than 15 sexual partners were also at risk for anal cancer (for heterosexual men: OR=3.9, for homosexual men: OR=6.6). These findings suggest that MSM can be considered a risk group for anal cancer.

Women with anal cancer were more likely to report a history of anal intercourse (16.9%) compared with women without anal cancer (11.0%). The risk for anal cancer was especially high in women who had more than 10 sexual partners.17,39 A history of sexually transmitted diseases is correlated with a higher risk for anal cancer.17,30,31 This is prone to confounding, since it is hard to distinguish from the increasing risk that is already caused by receptive anal intercourse.

**HIV-positive persons**

Immunosuppression is reported to be an important factor in the development of anal cancer. The association between anal cancer and HIV infection is difficult to confirm because of confounders. The relationship between anal cancer and receptive anal intercourse has been mentioned above. In addition, HIV-positive individuals are reported to be more often detected with an HPV infection than HIV-negative individuals and when infected, often with more than one subtype.19-35 On the other hand, patients infected with HPV are seen to have higher rates of HIV infections.36

HIV-positive persons are more likely to be detected with AIN,13-17,35-38 and have a more rapid progress from AIN to anal cancer. It is suggested that the greater risk for anal cancer in HIV-positive as compared with HIV-negative persons is caused by differences between HIV-positive and HIV-negative persons in the biology of anal HPV infection and anal cancer.19,39 Immunosuppression probably plays a role: a negative correlation was found between CD4+ T cell counts and the appearance of AIN13-17,40 and anal cancer40,41 in HIV-positive persons. There are also studies that suggest an independent correlation between anal cancer and HIV infection itself.42 A decrease of incidence has not been seen during the last years and it has been suggested that the widespread use of combination antiretroviral therapy (cART) makes HIV-positive individuals live longer and makes them prone to infection with HPV for a longer period, leaving more time for developing anal cancer. According to this explanation, anal cancer is more associated with a persistent HPV infection than with HIV.13-14,45

One study reported that even during an early stage of HIV infection the incidence of anal cancer was increased significantly. This finding also suggests that severe immunosuppression caused by the HIV infection is not the only explanation for the development of anal cancer.46 Two recent meta-analyses, combining data from six75 and eight individual studies respectively, showed an increased risk for anal cancer in HIV-infected patients of approximately 30-fold compared with the general population (SIR=28.75; 95% CI 21.6-38.3).67 (SIR=28; 95% CI 21-35).48 Before the introduction of cART in 1996, the incidence of anal cancer was 6.8-fold higher among HIV-positive women compared with the general population.49 HIV-infected MSM were reported to have the highest risk for anal cancer.49,49,51 A recent meta-analysis, including nine individual studies, revealed that the incidence of anal cancer among HIV-positive MSM was 46 per 100,000 person-years and therefore much higher (p=0.011) than the incidence among HIV-negative MSM, which was 5 per 100,000 person-years (figure 5). The incidence rate found in HIV-negative MSM is still higher than the incidence in the general population.50

**Organ transplant recipients**

Chronic immunosuppressive therapy, for example following solid organ transplantation, is known to be a risk factor for several types of squamous cell carcinomas. Since the most common type of anal cancer is squamous cell carcinoma, several studies have found a high risk for anal cancer for patients receiving immunosuppressive therapy.24-31 It is thought that the increased risk is the result of persistent HPV infection, caused by chronic immunosuppression.34,38

A recent meta-analysis in 2007 estimated the risk of anal cancer for solid organ transplant recipients at six times higher compared with the general population (SIR=5.85; 95% CI 1.36-17.3), based on two studies from Sweden and Australia/New Zealand.47 A Danish nationwide cohort study published in 2010 revealed a 14-fold higher risk for anal squamous cell carcinoma compared with the general Danish population (SIR=14.4; 95% CI 7.0-26.4),39 and a comprehensive cohort study (1987-2008) in the United States reported a sixfold higher risk compared with the US general population (SIR=5.84; 95% CI 4.7-7.18) (p value <0.001). The incidence of anal cancer among organ transplant recipients was 11.6 per 100,000 person-years according to this study.46

**Women with a history of cervical cancer or cervical intraepithelial neoplasia**

As mentioned above, population-based studies have found a link between cervical cancer and anal cancer.46 This link is explained by HPV infection.1 Women with anal cancer are more likely to have had a history of vulvar/vaginal cancer (OR=15.4; 95% CI 4.9-48.0) or cervical cancer (OR=4.3; 95% CI 2.7-6.9) according to data from the Danish Cancer Registry for the period 1943-1989.47
In a prospective population-based study of all Swedish women aged 18-50 years, the incidence rate ratios of anal cancer in women with a history of cervical intraepithelial neoplasia (CIN) grade 3 was investigated for the period 1968-2004. Women with such history had a fivefold higher risk for anal cancer. A more recent study in the United States using data from the SEER program from the period 1973-2007 reported a 16-fold higher risk for anal cancer for women with a history of cervical intraepithelial neoplasia and a sixfold higher risk for women with a history of cervical cancer. Women with histories of vulvar intraepithelial neoplasia and vulvar cancer had higher risks as well: SIR=22.2 and SIR=17.4, respectively. The increased risks could not be explained by therapeutic interventions for cervical cancer, such as radiation, in any of the studies.

**Figure 5.** Incidence of anal cancer in men who have sex with men, by HIV status and before and after the introduction of combination antiretroviral therapy (cART) (= HAART); derived from reference 35.
Incidence and trends in incidence of anal cancer among risk groups

The first literature search focusing on the incidence of anal cancer was used to identify articles on the specific incidence rates and trends in time of anal cancer among risk groups as well (figure 2). From this search, ten articles were useful concerning the HIV-positive persons and MSM, one concerning the specific incidence rates and trends in time of anal cancer among organ transplant recipients, and no articles were useful for the risk group of women with a history of cervical cancer or cervical intraepithelial neoplasia.

If significant changes can be seen in the incidence of anal cancer for these risk groups, this might (partly) explain the increasing incidence of anal cancer world-wide. Studies that describe trends in time in anal cancer incidence among these groups are rare. Therefore we also used an alternative way to determine whether a risk group contributes to the increase in anal cancer. The (increasing) incidence of anal cancer in a risk group can be estimated by a simple multiplication using the two factors increased risk for anal cancer (defined by standardised incidence ratios, for example) in that particular risk group and the increase in absolute number of persons in that risk group.

HIV-positive persons

A meta-analysis combining incidence data from four studies of the pre-cART era (before 1996) and five studies of the cART era (from 1996 onwards) showed that the standardised incidence ratio increased from 37 (95% CI 19-75) in the pre-cART era to 47 (95% CI 22-100) in the cART era.

A prospective cohort study in England, not included in this meta-analysis, found similar data. The standardised incidence ratio of anal cancer compared with the general population in this cohort, including 8640 HIV-positive patients, has risen from 35 per 100,000 person-years in the period before the introduction of cART (1984-1995) to 92 per 100,000 in the cART era (1996-2003) (p value >0.05), which is significantly higher than the incidence in the general population (p value <0.001 for both).

HIV-positive MSM

As discussed above, HIV-positive MSM are the most prominent risk group for anal cancer. One meta-analysis, investigating the incidence of anal cancer among HIV-positive MSM before and after the introduction of cART, combines data from nine individual studies (six HIV/AIDS and cancer registries linkage studies and three observational cohort studies). The incidence of anal cancer among HIV-positive MSM was higher from 1996 onwards (after introduction of cART) (78 per 100,000 person-years), than it was before 1996 (22 per 100,000) (p value=0.013) (figure 3). The authors of this meta-analysis remark that incidence rates of the pre-cART era are not age adjusted and some of the increase might be explained by ageing of the HIV-positive population.

One of the studies in the above meta-analysis, based on combined incidence data of 13 cohorts in North America from 1996 and 2007, reports a plateau phase in the increase of incidence among HIV-positive MSM for recent years. Standardised incidence ratios for HIV-positive MSM have developed from 90 per 100,000 in the period 1996-1999 to 159 per 100,000 in the period 2000-2003 and 131 per 100,000 in the period 2004-2007. For the Netherlands, we also observed such a plateau in incidence, with approximately 20 cases of anal cancer diagnosed annually in HIV-positive MSM (Richel O, unpublished data).

Since HIV-positive MSM have a 80-fold higher risk for anal cancer, an increase in the proportion of HIV-positive MSM in the population will contribute to a higher incidence of anal cancer in the general population. If we look further into the MSM population in the Netherlands, by means of the Monitoring Reports of the Dutch HIV Monitoring Foundation, we see that the HIV-infected MSM population in follow-up has increased by 51.7% from 5619 in 2007 to 8523 in 2011. This means that the proportion of HIV-positive MSM in the population is increasing over time. Registration differed for the years before 2007.

Organ transplant recipients

No studies on the trend in time in incidence of anal cancer among organ transplant recipients could be found. As already discussed, organ transplant recipients have a much higher risk for anal cancer compared with the general population. If the proportion of organ transplant recipients in the population increases, this is likely to increase the incidence of anal cancer. A recent Dutch population-based retrospective cohort study, based on data from the Dutch Foundation for Renal Replacement Therapy Registration (Renine), showed that the number of renal transplant recipients increased in the period 1995-2009, from 1640 renal transplant recipients in 1995 to 8400 recipients in 2009.

Discussion

Incidence rates have increased in practically all Western countries during the last decades. Whereas infection with oncogenic HPV is the most important aetiological factor, several risk factors and risk groups for anal cancer have been identified during the last decennia, in particular smoking (OR=3.9-5 for men, OR=3.8 for women), MSM (OR=17.3), MSM practising receptive anal intercourse (additional OR=6.8), a history of sexually transmitted diseases, having had more than 15 sexual
partners, HIV positivity (OR=28.28.75), HIV-positive MSM (SIR=77.8), organ transplant recipients (SIR=5.8) and women with a history of cervical cancer (SIR=6.2) or cervical intraepithelial neoplasia (SIR=16.4). We showed that incidence rates of anal cancer among HIV-positive persons have significantly increased over time in multiple countries. Such data are not available for other risk groups. The increasing incidence of anal cancer could be caused by an increased risk for anal cancer in specific risk groups and in addition by increasing numbers of patients belonging to these risk groups. If the absolute number of these risk groups in the population increases, this is likely to contribute to the increasing incidence of anal cancer. We found that the number of HIV-positive MSM in the Dutch population increased by 51.7% from 2007 to 2011. Since HIV-positive MSM have a significant risk for anal cancer, this supports our hypothesis that this risk group contributes to the overall increase in incidence. HIV-positive MSM account for approximately 50% (20 deaths per year) of the total of 40 people dying annually from anal cancer in the Netherlands. The increased number of renal transplant recipients probably contributes to the increased incidence of anal cancer as well: in the Netherlands, the number of renal transplant recipients increased from 3640 in 1995 to 8400 in 2009. This probably also applies to other countries. Other factors are also likely to influence the incidence of anal cancer. In the Netherlands, for example, the number of people smoking cigarettes decreased by 20% from 2000 through 2007. Based on these figures one would expect the incidence of anal cancer to decrease, since smokers have a three- to fivefold increased risk for anal cancer.

Limitations of this review mostly result from limitations in the studies used in this review. One of the limitations is the difference in defining anal cancer between studies from individual countries. Most, but not all, studies used identical topographical codes from the International Classification of Diseases for Oncology (ICD) to classify cancer of the anus, anal canal, and anorectum (C21.1-C21.8). Another limitation is that distinction between histological types of anal cancer (e.g. squamous cell carcinoma or adenocarcinoma) was not always made in the articles we used. Furthermore, the studies reported incidence data for different periods of time and used different standard populations for age adjustment. Therefore, direct comparison of the results was sometimes difficult. One factor that should be considered is the use of more and/or better diagnostic methods for the detection of anal cancer over time. However, studies that support this suggestion have not been found. In conclusion, we have shown that an increased risk for anal cancer in certain risk groups, in particular HIV-positive MSM and organ transplant recipients, and increasing numbers of people belonging to these risk groups contributes to the overall increase in anal cancer incidence. Further studies should answer the question to what exact extent these risk groups contribute to the overall anal cancer incidence, and whether these risk groups would benefit from preventive screening for anal cancer.

REFERENCES


ABSTRACT

Hepcidin inhibits the iron export from duodenal cells and liver cells into the plasma and therefore plays a key role in controlling iron homeostasis. In obese patients, elevated cytokine production stimulates hepcidin synthesis, causing iron to be retained as ferritin in e.g. macrophages (functional iron deficiency). In addition, patients often develop iron deficiency after bariatric surgery due to malabsorption, which may cause anaemia and thereby lead to complaints such as fatigue. In these patients, the absorption of iron may be disrupted because the reduction of Fe³⁺ by gastric acid into Fe²⁺ (the form that is easily absorbed) is not so effective after stomach reduction. Iron absorption is further reduced after malabsorptive interventions as a result of bypassing the duodenum and the proximal part of the small intestine, where the absorption takes place. Oral iron supplements often have little effect after bariatric surgery. Intravenous supplements of iron can restore the iron status rapidly after bariatric surgery, resulting in fewer symptoms such as fatigue.

KEYWORDS

Anaemia, bariatric surgery, hepcidin, intravenous iron supplementation, iron deficiency

INTRODUCTION

Over the past few years, there has been a strong increase in the number of bariatric operations performed, because of the annual increase in the incidence of morbid obesity (BMI >40 kg/m²). In 2011, the Netherlands Association of Surgeons drew up a new guideline for the treatment of ‘morbid obesity’. A review of the role of bariatric surgery in reducing this threat to public health was recently published in this journal.

The absorption of essential vitamins and minerals is often disrupted after bariatric surgery. The problem of iron deficiency in particular is often underestimated. In recent years, new insights have been gained into iron homeostasis and the prevalence, diagnosis and treatment options for iron deficiency related to bariatric procedures. We carried out a literature study in PubMed about this, looking at studies on the subject that have been published over the last five years. This article contains a summary of these studies.

VARIOUS BARIATRIC PROCEDURES

There is a wide range of bariatric procedures and they are generally classified by their assumed mechanism:

- Restrictive procedures: based on limiting the intake of food by gastric reduction, such as sleeve gastrectomy.
- Malabsorptive interventions: based on limiting absorption of nutrients by biliopancreatic diversion (BPD): division of part of the small intestine into two branches, one nutrient branch to transport nutrients and one biliopancreatic branch to transport biliopancreatic juices. This bypass only makes it possible to absorb food via the small remaining common part of the small intestine.
- Combined restrictive and malabsorptive interventions, such as a Roux-en-Y gastric bypass (RYGB), combining gastric reduction and biliopancreatic diversion.
More information about these types of bariatric surgery can be found in the previously mentioned review.²

IRON DEFICIENCY ANAEMIA RELATED TO BARIATRIC SURGERY

Prevalence of iron deficiency anaemia before and after bariatric surgery
Anaemia, iron deficiency or a combination of the two often develops after a patient has undergone bariatric surgery. The prevalence figures for anaemia and iron deficiency in 1252 morbidly obese patients before bariatric surgery were 14% and 28% respectively.² Postoperatively, these percentages were seen to increase to 30-60% for iron deficiency and anaemia, depending on the definition of iron deficiency, the duration of the follow-up period, the type of intervention, the patient population, and supplementation of iron.³ This corresponds to the prevalence figures for anaemia from a large American cohort study (n=1125) of bariatric patients: 12% preoperatively and 23% postoperatively.⁴ Table 1 presents various risk factors for developing iron deficiency and/or iron deficiency anaemia.

Figure 1 shows the results of a Dutch study into the prevalence of anaemia and iron deficiency in 377 patients before and after weight-loss surgery (RYGB).⁵ The percentage of iron deficiency anaemia one year after the operation was 16%. Another 16% of patients showed iron deficiency (without anaemia) and might develop anaemia if no iron supplementation is given.⁵ A drawback of this study is that serum ferritin levels were not measured, the method that has gained widest acceptance as an indicator of iron status.⁶

New insights into the regulation of iron status
Iron homeostasis (figure 2) balances out the iron demands of various organs and ensures efficient reuse of iron from macrophages, as well as controlled storage of iron. Most of the iron in our bodies can be found together with

Table 1. Risk groups and risk factors for developing ID and anaemia before and after bariatric surgery
- Women⁵,¹⁸ and in particular:
  - premenopausal women¹³,¹⁴
  - pregnant women¹³
- Adolescents:¹³,¹⁸
- BMI >50 kg/m²³
- Vitamin B₁₂ deficiency¹⁸
- After malabsorptive interventions (in the long term, the risk is greater than for restrictive interventions):³,¹⁷

Figure 1. Prevalence of anaemia*, iron deficiency anaemia (IDA) and iron deficiency* (ID) before and one year after malabsorpive bariatric surgery (RYGB). Figure is based on data from a Dutch study into prevalence of anaemia and related deficiencies (n=377).

Figure 2. Iron homeostasis with hepcidin as the regulator. Storage of iron in red blood cells (RBC), liver, spleen, macrophages and bone marrow.⁶

Fpn = ferroportin: release of iron to plasma; Fe-Tf = iron-transferrin complex: transport protein for the iron, RBC = red blood cell.
protoporphyrin in the core of haem, which is used to make haemoglobin in red blood cells and is responsible inter alia for oxygen and carbon dioxide transport. When red blood cells are broken down by macrophages, their iron is extracted and stored for reuse.7,8 The hormone hepcidin plays a key role in maintaining a balance between iron deficiency on the one hand and accumulation of iron on the other (figures 2 and 3). Hepcidin is produced in the liver and binds to the membrane-bound iron export protein ferroportin, which can be found in duodenal cells, among others. Ferroportin is deactivated after binding: iron can then temporarily not be released into the plasma. The iron is then stored as ferritin in the duodenal cell and the absorption of iron from within the intestinal lumen is inhibited.9 If hepcidin production is low, the iron stored will be released to the plasma as needed and transported in the form of transferrin-bound iron to the cells that need iron. Increased erythropoiesis or reduced iron plasma levels inhibit the production of hepcidin, which increases iron release to the plasma. Accumulation of iron and inflammatory processes stimulate the synthesis of hepcidin, thus inhibiting the release of iron to the plasma. As a result, iron remains locked in the duodenal cells and will largely be lost in the faeces after the intestinal cell itself is lost.79

Causes and consequences of iron deficiency/iron deficiency anaemia

Causes of iron deficiency and iron deficiency anaemia
Iron deficiency can develop if iron metabolism is disturbed. Causes of such a disturbance include haemorrhage, insufficient iron absorption from the food in the duodenum or increased hepcidin concentrations caused by, for example, inflammation.

Over recent years, more insights have been gained into the relationship between obesity and iron levels. Cytokines such as interleukin-6 and TNF-α are produced in fatty tissues, inducing an inflammatory response. Activation of the immune system then results in increased hepcidin production, followed by reduction of iron levels in the plasma and an increase in ferritin (functional iron deficiency). As a result, the production of haemoglobin will be disturbed and anaemia develops.3-9 In addition to their nutritional patterns, this explains the relatively high percentages of iron deficiency and iron deficiency anaemia in patients with morbid obesity prior to bariatric surgery.3,10,12

Bariatric surgery often results in drastic weight loss.4 In some studies, this weight loss was associated with reduction in the inflammatory status, with recovery from the functional iron deficiency as a result.10-12 However, iron deficiency and/or anaemia are observed in most studies in the follow-up period after the bariatric procedure (see above). These mostly referred to malabsorptive interventions. In addition to gastrointestinal blood loss, iron absorption can also be disturbed for various other reasons. As a result of the stomach reduction, non-haem bound Fe³⁺ cannot be reduced effectively by gastric acid to the more easily absorbed Fe²⁺.5,13 The uptake of haem iron is disrupted too. The bypass delays the interaction with biliopancreatic juices. It is then more difficult for haem to be released from myoglobin and haemoglobin.13 In addition, eating red meat (the major source of haem iron) is less well tolerated after stomach reduction.5,13 Finally, iron is mainly absorbed in the duodenum and proximal jejunum.7 This absorption cannot take place because this part of the small intestine has been bypassed.13-15 The length of the remaining part of the jejunum, now used as a branch for transporting food, hardly affects the iron absorption. This suggests that there is no up-regulation of iron absorption further along the small intestine, which could have compensated for the duodenal bypass.13 However, this type of up-regulation does occur for some other nutrients, such as folate.3,5

Consequences of iron deficiency and iron deficiency anaemia
Iron deficiency inhibits the synthesis of haemoglobin and may thereby cause anaemia. This often causes severe fatigue,3,4 which is undesirable for patients for whom a healthy lifestyle with a lot of physical exercise is very important. In addition to the deficient oxygen transport caused by the anaemia, iron deficiency may also lead to disruption of cell division, myelination, cellular immune response and the oxidative metabolism.7 This may also become manifest in all kinds of symptoms. Iron deficiency may result in fatigue symptoms without anaemia being
involved, and patients with functional iron deficiency often suffer long periods of systemic inflammation.3

**DIAGNOSIS AND TREATMENT OF IRON DEFICIENCY AND IRON DEFICIENCY ANAEMIA**

Diagnosis of iron deficiency and iron deficiency anaemia Before bariatric surgery and for a long period thereafter, it is important to monitor the nutritional status of the patient. The possibility of iron deficiency is one thing to look out for during monitoring. The classic indicators of iron deficiency are serum ferritin ↓, serum iron ↓, TIBC ↑ (=total iron-binding capacity) or TSAT ↓ (transferrin saturation), and serum Hb ↓.13 The possibility of functional iron deficiency can best be detected by measuring the TSAT. After all, ferritin (stored iron) may be elevated if obesity or inflammation is involved, as a result of which relatively high iron levels can be interpreted wrongly.3 After bariatric surgery, the serum ferritin can in fact be an early indicator of disturbed iron absorption, as the iron stock is used up first before the TSAT decreases, after which the haemoglobin production decreases too.13

**Treatment options for iron deficiency and iron deficiency anaemia**

Iron deficiency can be treated with oral or parenteral iron supplements. The disadvantages of oral treatment are the disputed effectiveness, the long period before effects are experienced1 and the poor therapy compliance, as well as gastrointestinal side effects (such as nausea and constipation).5 In addition, the release of iron from duodenal cells to the plasma is disturbed in patients who are severely overweight (see above). After malabsorptive interventions, the duodenal bypass reduces absorption of orally administered iron (see above). Oral iron supplements are therefore often ineffective for treating iron deficiency and iron deficiency anaemia after such bariatric interventions.6,8,14-16,18 A recent study showed that sufficient absorption of orally administered iron sulphate after RYGB surgery was observed in only one patient out of a group of 11.18 According to European guidelines, oral supplementation of micronutrients after bariatric surgery should only be used in a preventive regimen.19

Parenteral iron supplements are therefore recommended for correction of iron deficiency or iron deficiency anaemia after bariatric surgery.19 The disadvantages of intramuscular administration are the painful injection and skin discoloration.15 The most important advantage of intravenous iron is the rapid correction of iron deficiency and iron deficiency anaemia, and the fact that transport through the intestinal mucous membranes is avoided. The iron-polysaccharide complex is absorbed by the macrophages after parenteral administration.19 A relatively new intravenous iron preparation has been developed, because of the limitations associated with iron dextran and iron sucrose.20 Ferric carboxymaltose (FCM) avoids dextran-inducing hypersensitivity reactions and overcomes

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<td>Intravenous FCM or placebo (n=290 premenopausal women)</td>
<td>Ferritin; TS, PFS score</td>
<td>FCM vs placebo: day 56 ↓ PFS score: 35% versus 19% (p&lt;0.001) ferritin recovery: 99 vs 2%</td>
<td>Well tolerated</td>
</tr>
</tbody>
</table>

*11 randomised studies (including 3 double-blind placebo-controlled studies) and 3 cohort studies; **Hb increase ≥2g/dl (primary endpoint); FCM = ferric carboxymaltose; IBD = inflammatory bowel disease; IS = iron sucrose; PFS = Piper Fatigue Scale; (S)AEs = (serious) adverse events; TS = transferrin saturation.

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**Table 2. Studies into the effects of intravenous iron (FCM; Ferinject) in iron deficiency (ID) and iron deficiency anaemia (IDA)**

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the low-dosage limitations of iron sucrose. In contrast to the older preparations, FCM can be administered in one go (a maximum of 1000 mg iron) by rapid infusion and has a neutral pH and a physiological osmolarity. It is therefore a highly stable complex that does not release ionic iron under physiological conditions and does not provoke oxidative stress reactions. These characteristics mean that a test dose is not required beforehand and that FCM is relatively safe and offers convenience for patients.4

Numerous comparative clinical studies have now been performed for FCM, showing rapid correction of iron deficiency and a reduction of symptoms (Table 2).

So far, there have been very few studies into the effect of intravenous supplements of iron after bariatric surgery. A retrospective analysis studied the effect of intravenous supplements of iron after bariatric surgery.14 These patients, who had been given oral iron supplements without success, responded successfully to intravenous iron supplements and their anaemia was alleviated. The frequency of intravenous iron supplements varied from once every four months to once every 11 months. In addition, various case reports have described how intravenous supplementation of iron in anaemic patients after bariatric surgery resulted in rapid and complete recovery of Hb values and iron parameters.5

Recommended iron supplementation in bariatric surgery

Over the last few years, iron supplements for bariatric patients have come to be seen as increasingly important. Any preoperative deficiency should be treated first, before surgery is performed. Iron levels should be checked regularly postoperatively. Because iron deficiency can develop years after bariatric surgery, lifelong checks are recommended.15

There are no specific guidelines in the Netherlands for treating iron deficiency and anaemia in bariatric patients. Table 3 therefore contains an overview of recommendations for such treatment. It has been drawn up on the basis of the literature summary and the European guidelines on surgery for severe obesity.19 Iron supplements can be administered orally as a preventive policy. Usually, oral supplements are ineffective if the iron deficiency existed beforehand or is present after the bariatric surgery. Intravenous iron administration has a more rapid effect, causes fewer side effects and circumvents the transport from the intestinal mucous membranes to the plasma. New intravenous iron preparations such as iron carboxymaltose are relatively safe, easy to use (one short infusion per treatment) and therefore cost-effective.3

<table>
<thead>
<tr>
<th>Blood values</th>
<th>Prevention and treatment of ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operative</td>
<td>Iron, ferritin, TSAT, vitamin B₁₂, folate</td>
</tr>
<tr>
<td>Post-operative</td>
<td>During checks*: iron, ferritin, TSAT, vitamin B₁₂, folate</td>
</tr>
<tr>
<td></td>
<td>If necessary: calcium, vitamin D, copper, selenium and protein</td>
</tr>
<tr>
<td></td>
<td>In the event of symptoms: also Hb, MCV</td>
</tr>
</tbody>
</table>

*Regular checks: e.g. every 3 months during 1st year; every 6 months 2nd year and every year thereafter

ID = iron deficiency; IDA = iron deficiency anaemia; MCV = mean corpuscular volume; TSAT = transferrin saturation.

CONCLUSION

Morbid obesity often leads to functional iron deficiency developing because increased cytokine production disturbs the transport from the intestinal mucous membranes to the plasma. In addition, bypassing the proximal part of the small intestine in malabsorptive bariatric interventions prevents iron absorption in that part of the small intestine. This explains why oral iron supplements are often ineffective for correcting iron deficiency and iron deficiency anaemia both before and after bariatric surgery. Intravenous iron administration has a more rapid effect, causes fewer side effects and circumvents the transportation from the intestinal mucous membranes to the plasma.

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The aetiology of community-acquired pneumonia and implications for patient management

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ABSTRACT

Purpose: Understanding which pathogens are associated with clinical manifestation of community-acquired pneumonia (CAP) is important to optimise treatment. We performed a study on the aetiology of CAP and assessed possible implications for patient management in the Netherlands.

Methods: Patients with CAP attending the emergency department of a general hospital were invited to participate in the study. We used an extensive combination of microbiological techniques to determine recent infection with respiratory pathogens. Furthermore, we collected data on clinical parameters and potential risk factors.

Results: From November 2007 through January 2010, 339 patients were included. Single bacterial infection was found in 39% of these patients, single viral infection in 12%, and mixed bacterial-viral infection in 11%. Streptococcus pneumoniae was the most frequently identified pathogen (22%; n=74). Infection with atypical bacteria was detected in 69 (20%) of the patients.

Conclusion: Initial empirical antibiotics should be effective against S. pneumoniae, the most common pathogen identified in CAP patients. The large proportion of patients with infection with atypical bacteria points to the need for improved diagnostic algorithms including atypical bacteria, especially since these atypical bacteria are not covered by the first-choice antibiotic treatment according to the recently revised Dutch guidelines on the management of CAP.

KEYWORDS

Antibiotic treatment, clinical diagnosis, community-acquired pneumonia

INTRODUCTION

Pneumonia is a common and potentially serious respiratory disease, mainly in children and the elderly. It is the third most common cause of death worldwide.1-5 Community-acquired pneumonia (CAP) is defined as an acute symptomatic infection of the lower respiratory tract which develops outside the hospital or nursing home, and whereby a new infiltrate is demonstrated on chest X-ray.5 Several studies show that Streptococcus pneumoniae is the predominant aetiological agent of CAP, followed by other bacteria such as Haemophilus influenzae, Staphylococcus aureus, Chlamydia pneumoniae, Legionella spp, and Mycoplasma pneumoniae.1,7-12

Recent developments in molecular diagnostics have resulted in increased detection of respiratory viruses, including influenza virus, para-influenza virus and respiratory syncytial virus (RSV), in patients with pneumonia.2,3,17 Reported studies on the aetiology of CAP diverge with respect to the proportions of detected pathogens as well as the diagnostic deficit, i.e. the proportion of patients for which no pathogen could be detected. These discrepancies are attributable to differences in the epidemiology of pathogens, study population, available patient specimens and the diagnostic methods used.
Changes in the aetiology of CAP and in bacterial antibiotic-resistance patterns over time might have important implications for patient management. However, in clinical practice, causative pathogens remain unknown in the majority of CAP patients, since microbiological tests are not used or are limited to blood and sputum cultures for bacterial causes. Most patients are therefore treated empirically. Until recently, initial therapy with amoxicillin or doxycycline was recommended for patients with mild CAP in the Netherlands. Due to the increasing resistance of S. pneumoniae to doxycycline, amoxicillin is now assigned as first-choice treatment.

To optimise future treatment choices, clear understanding of respiratory pathogens in relation to the clinical manifestation of CAP is important. We therefore performed a prospective observational study on the aetiology of CAP in the Netherlands, using an extensive combination of microbiological and molecular techniques. Furthermore, we studied potential relations between the type of identified pathogen and specific host factors, comorbidity, and known risk factors for CAP.

MATERIAL AND METHODS

Study population
Patients attending the emergency department of the Jeroen Bosch Hospital (JBH) in ’s-Hertogenbosch, serving a rural area in the Netherlands covering about 2% of the Dutch population, with CAP were invited to participate in the study during the time period November 2007 through January 2010. CAP is defined as an acute lower respiratory tract infection with at least two of the following clinical symptoms: new onset of cough, sputum production or change in colour of respiratory secretions in patients with chronic cough, fever or hypothermia or physical examination consistent with pneumonia, whereby a new infiltrate is demonstrated on chest X-ray. Exclusion criteria were age <18 years, being transferred from another hospital and being a nursing home resident. Ethical approval for the study was obtained from the Medical Ethical Review Committee (Tilburg, the Netherlands) and informed consent was obtained from all included patients.

At hospital admission, the attending physician or triage nurse used Case Report Forms (CRFs) to report data on: 1) patient characteristics, 2) use of antibiotics in the past two weeks, 3) influenza vaccination status, 4) comorbidity: COPD, congestive heart failure, malignancy, immune deficiency, renal disease, liver failure and cerebrovascular disease, 5) potential risk factors for CAP, such as influenza-like illness (ILI) in the past four weeks and smoking status, 6) specific medical history and physical examination, and 7) Pneumonia Severity Index (PSI) and CURB-65 score.

Laboratory analyses
Blood, sputum and urine specimens, as well as combined nose and throat swabs, were obtained at hospital admission. Sera for paired serology were collected at acute (day of hospital admission) and convalescence phases (>28 days after hospital admission). Microbiological testing (table 1) was performed both at the JBH and the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands). All collected specimens were sent immediately to the JBH laboratory. Subsequently, EDTA blood specimens for S. pneumoniae PCR were sent to the RIVM within 48 hours after sampling. Following routine microbiological testing at JBH, the remaining serum and sputum specimens were stored at -20°C and -80°C, respectively. Immediately after sampling, combined nose and throat swabs were added to a virus transport medium (VTM). The swab was thoroughly vortexed in the VTM, and the VTM was distributed to three ampoules. One of these was used for diagnostic investigation at JBH and the other two were stored at -80°C. Urine specimens for detection of S. pneumoniae capsular polysaccharide were stored at -80°C. Periodically, the serum, sputum and urine samples as well as the VTM from the combined nose and throat swabs were transported to the RIVM for additional study-specific microbiological testing. Before performing additional tests at the RIVM, the sputum specimens were pre-treated using the MagNA Lyser (Roche) instrument to disrupt the sputum before nucleic acid extraction.

Statistical analyses
The data were entered in a Microsoft Access database and analysed using SAS software, version 9.3 (SAS Institute Inc., Cary, NC, USA).

Descriptive statistics were calculated, results of categorical variables are presented as percentages and continuous variables as median with range. Patients were grouped into four categories based on the results of blood culture, sputum culture, PCR, rapid antigen detection test, and rise in titre in paired sera. In case of infection with S. pneumoniae, H. influenzae, Moraxella catarrhalis, S. aureus, Viridans streptococci, Escherichia coli, Enterococcus spp., Haemophilus parainfluenzae, Klebsiella pneumoniae, Pseudomonas aeruginosa, group A beta-haemolytic streptococci or group B beta-haemolytic streptococci, patients were assigned to the group ‘typical bacteria’(1). In case of infection with Coxiella burnetii, M. pneumoniae, Legionella pneumophila, Chlamydia psittaci or C. pneumoniae, but without infection with the earlier mentioned (typical) bacteria, patients were assigned to the group ‘atypical bacteria’(2). Patients with only viral infection were classified as ‘only viral pathogens’(3) and the remaining patients were assigned to the group ‘no pathogens’(4). Furthermore, we distinguished between patients with simultaneous bacterial and viral infection.

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Table 1. Microbiological techniques performed on different samples at Jeroen Bosch Hospital ('s-Hertogenbosch, the Netherlands - JBH) and the National Institute for Public Health and the Environment (Bilthoven, the Netherlands – RIVM)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Microbiological technique</th>
<th>Micro-organism</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood EDTA blood Serum</td>
<td>PCR</td>
<td>Among others <em>S. pneumoniae</em>, group A beta-haemolytic streptococci, group B beta-haemolytic streptococci, <em>Haemophilus influenzae</em>, <em>Staphylococcus aureus</em>, <em>Pseudomonas aeruginosa</em>, Enterococcus spp, anaerobic bacteria</td>
<td>JBH</td>
</tr>
<tr>
<td></td>
<td>Serology</td>
<td><em>Mycoplasma pneumoniae</em>, <em>Chlamydia psittaci</em>, <em>Legionella pneumophila 1-6</em>, <em>Coxiella burnetii</em></td>
<td>JBH</td>
</tr>
<tr>
<td>Sputum</td>
<td>PCR</td>
<td><em>C. burnetii</em>, <em>L. pneumophila</em></td>
<td>JBH</td>
</tr>
<tr>
<td></td>
<td>Bacterial culture a gram preparation</td>
<td>Among others <em>S. pneumoniae</em>, group A beta-haemolytic streptococci, group B beta-haemolytic streptococci, <em>H. influenzae</em>, <em>Haemophilus parainfluenzae</em>, <em>Moraxella catarrhalis</em>, <em>S. aureus</em>, <em>P. aeruginosa</em>, Enterococcus spp, anaerobic bacteria</td>
<td>JBH</td>
</tr>
<tr>
<td>Combined nose and throat swab</td>
<td>PCR</td>
<td><em>L. pneumophila</em></td>
<td>JBH</td>
</tr>
<tr>
<td></td>
<td>PCRam</td>
<td><em>M. pneumoniae</em>, <em>Chlamydia pneumoniae</em>, influenza viruses, parainfluenza viruses, respiratory syncytial virus, coronaviruses, rhinovirus, adenovirus, enterovirus, human metapneumovirus</td>
<td>RIVM</td>
</tr>
<tr>
<td>Urine</td>
<td>PCR</td>
<td><em>L. pneumophila</em></td>
<td>JBH</td>
</tr>
<tr>
<td></td>
<td>PCRam</td>
<td><em>M. pneumoniae</em>, <em>Chlamydia pneumoniae</em>, influenza viruses, parainfluenza viruses, respiratory syncytial virus, coronaviruses, rhinovirus, adenovirus, enterovirus, human metapneumovirus</td>
<td>RIVM</td>
</tr>
<tr>
<td></td>
<td>Rapid antigen detection</td>
<td><em>L. pneumophila serogroup 1, S. pneumoniae</em></td>
<td>JBH</td>
</tr>
<tr>
<td></td>
<td>Capsular polysaccharide identification</td>
<td><em>S. pneumoniae</em></td>
<td>RIVM</td>
</tr>
</tbody>
</table>

*Coronavirus types OC43, NL63 and 229E.

(mixed bacterial-viral infections) and patients with only bacterial or only viral infection (single infections). We used multivariate logistic regression analysis to examine whether gender, age ≥70 years, comorbidity, use of antibiotics in the past two weeks, ILI in the past four weeks, and smoking behaviour were associated with the type of infection detected (dependent variable). Variables with a p value ≤0.20 in the univariate model were included in the multivariate model. Backward selection was used to identify covariates that were independently associated with type of infection. Odds ratios (OR) were presented with 95% confidence intervals (CI).

**Results**

Aetiology

In the period November 2007 through January 2010, a total of 339 patients meeting the study criteria were included. We were able to detect infection with one or more bacterial pathogens for 168 (50%) of the 339 patients (table 2). Furthermore, we assessed viral infection for 77 (23%) of the 339 patients (table 3). For 37 (11%) of the 339 patients both bacterial and viral infection was assessed. Despite the use of an extensive combination of microbiological and molecular techniques, no infection could be determined for 131 (39%) of the 339 patients.

The most frequently identified bacterial pathogen was *S. pneumoniae* (22%), followed by *C. burnetii* (14%), *M. pneumoniae* (6%) and *H. influenzae* (4%). Mixed bacterial-viral infection was found for 25 (34%) of the 74 patients with *S. pneumoniae* infection, for seven (35%) of the 20 patients with *M. pneumoniae* infection and for five (33%) of the patients with *H. influenzae* infection, compared with only two (4%) of the 48 patients with *C. burnetii* infection. Rhinovirus was the most commonly identified viral pathogen (9%), followed by influenza virus type A (5%) and human metapneumovirus (hMPV; 4%). For 19 (66%) of the 29 patients with rhinovirus infection simultaneous bacterial infections were detected. For hMPV and influenza virus type A these numbers were six (46%) of the 13 and three (33%) of the 13, respectively.

Patient characteristics

Based on the identified infections, patients were categorized into the categories: 1) typical bacteria (n=99); 2) atypical bacteria (n=69); 3) only viral pathogens (n=40); and 4) no pathogens (n=131). Characteristics of these categories...

For patients with infection with atypical bacteria, comorbidity was reported for 13 (19%) of the 69 patients, compared with 130 (49%) of the 268 remaining patients. Antibiotic use prior to hospitalisation was reported for 48% of the patients. The most commonly used types of antibiotics were penicillins (56%), followed by tetracyclines (30%). Prior use of penicillins, which are considered not effective against some of the atypical bacteria such as C. burnetii, was remarkably often reported in patients with infection with atypical bacteria (74%). Severe pneumonia, i.e. PSI risk class IV-V and/or CURB-score ≥2, was reported for 20 (32%) of the 62 patients with infection with atypical bacteria, compared with 146 (61%) of the 238 other patients.

These observations were confirmed in multivariate logistic regression analyses that showed age ≥70 years (OR 1.8; 95% CI 1.1-3.3) and prior ILI (OR 3.5; 95% CI 1.2-9.8) to be significantly more common among CAP patients with viral infection compared with patients with bacterial infection. Comparing CAP patients with infection with atypical bacteria to patients with infection with typical bacteria showed that patients with ‘atypical pneumonia’ had used antibiotics significantly more often before hospitalisation (OR 2.4; 95% CI 1.1-5.2) and had less comorbidity (OR 0.2; 95% CI 0.07-0.4). When infection with atypical bacteria was limited to M. pneumoniae, L. pneumophila, C. psittaci and C. pneumoniae we found that patients with ‘atypical

are summarised in table 4. The category of atypical bacteria stands out with the relatively low median age, high proportion of males, low proportion of comorbidity, and high prior antibiotic use. The median age of the 69 patients with infection with atypical bacteria was 59 years (range: 18-96 years) and 77% were male, compared with 67 years (range: 20-96 years) and 59% male in the 268 remaining patients. Figure 1 shows the age distribution of the study population by the type of identified pathogen. Comorbidity was reported for 42% of the patients, with COPD as most frequently reported underlying disorder (32%). For patients with infection with atypical bacteria,
Table 4. Baseline characteristics of the patients with community-acquired pneumonia (CAP) attending the emergency room of a general hospital grouped by the type of detected pathogen, November 2007-January 2010

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Typical bacteria n=99</th>
<th>Atypical bacteria n=69</th>
<th>Viral pathogens n=40</th>
<th>No pathogens n=31</th>
<th>All patients n=339</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) of patients†</td>
<td>No. (%) of patients‡</td>
<td>No. (%) of patients§</td>
<td>No. (%) of patients∥</td>
<td>No. (%) of patients¶</td>
</tr>
<tr>
<td>Distribution by age group</td>
<td>&lt;50 years</td>
<td>(20.2)</td>
<td>(36.2)</td>
<td>(10.0)</td>
<td>(24.4)</td>
</tr>
<tr>
<td></td>
<td>(25)</td>
<td>(19)</td>
<td>(17)</td>
<td>(17)</td>
<td>(57)</td>
</tr>
<tr>
<td></td>
<td>&gt;50 and &lt;70 years</td>
<td>(43.4)</td>
<td>(39.3)</td>
<td>(47.5)</td>
<td>(32.1)</td>
</tr>
<tr>
<td></td>
<td>(37)</td>
<td>(19)</td>
<td>(42.5)</td>
<td>(43.5)</td>
<td>(127)</td>
</tr>
<tr>
<td></td>
<td>&gt;70 years</td>
<td>(16.4)</td>
<td>(24.6)</td>
<td>(43.5)</td>
<td>(57.5)</td>
</tr>
<tr>
<td>Male gender</td>
<td>55 (59.6)</td>
<td>53 (76.8)</td>
<td>23 (57.5)</td>
<td>77 (58.2)</td>
<td>212 (62.5)</td>
</tr>
<tr>
<td>Comorbidity†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>41 (51.1)</td>
<td>5 (8.3)</td>
<td>13 (37.1)</td>
<td>33 (28.2)</td>
<td>92 (21.5)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>10 (11.0)</td>
<td>4 (6.7)</td>
<td>3 (8.3)</td>
<td>8 (7.0)</td>
<td>25 (8.4)</td>
</tr>
<tr>
<td>Renal disease</td>
<td>9 (10.6)</td>
<td>2 (3.2)</td>
<td>3 (7.7)</td>
<td>10 (8.4)</td>
<td>24 (7.9)</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>7 (8.1)</td>
<td>4 (6.3)</td>
<td>7 (17.9)</td>
<td>14 (11.8)</td>
<td>32 (10.4)</td>
</tr>
<tr>
<td>Immune deficiency</td>
<td>2 (2.4)</td>
<td>2 (3.3)</td>
<td>1 (2.6)</td>
<td>6 (5.1)</td>
<td>11 (3.7)</td>
</tr>
<tr>
<td>Congenital heart defect</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Liver failure</td>
<td>54 (55.7)</td>
<td>13 (18.8)</td>
<td>20 (50.0)</td>
<td>56 (42.8)</td>
<td>134 (42.4)</td>
</tr>
<tr>
<td>Smoking</td>
<td>29 (40.3)</td>
<td>34 (57.6)</td>
<td>16 (48.5)</td>
<td>52 (47.3)</td>
<td>131 (47.8)</td>
</tr>
<tr>
<td>Type of antibiotic†‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillins</td>
<td>12 (41.4)</td>
<td>25 (73.5)</td>
<td>8 (50.0)</td>
<td>27 (54.0)</td>
<td>72 (55.8)</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>7 (24.1)</td>
<td>8 (23.1)</td>
<td>6 (37.5)</td>
<td>17 (34.0)</td>
<td>38 (29.8)</td>
</tr>
<tr>
<td>Quinolones</td>
<td>7 (24.1)</td>
<td>2 (5.9)</td>
<td>-</td>
<td>2 (4.0)</td>
<td>11 (8.5)</td>
</tr>
<tr>
<td>Other</td>
<td>8 (27.3)</td>
<td>3 (8.8)</td>
<td>3 (18.8)</td>
<td>7 (14.0)</td>
<td>21 (16.3)</td>
</tr>
<tr>
<td>Prior influenza-like illness†</td>
<td>7 (11.1)</td>
<td>5 (10.4)</td>
<td>8 (28.6)</td>
<td>16 (18.2)</td>
<td>36 (15.9)</td>
</tr>
<tr>
<td>Smoking</td>
<td>31 (39.7)</td>
<td>24 (44.4)</td>
<td>9 (27.3)</td>
<td>29 (28.2)</td>
<td>93 (31.4)</td>
</tr>
</tbody>
</table>

Distribution according to PSI score†‡: t1-3 I-III 55 (64.7) 48 (77.4) 19 (54.3) 69 (59.3) 191 (64.1)  IV 22 (25.0) 8 (12.9) 11 (31.4) 17 (33.6) 80 (26.8)  V 8 (9.4) 6 (9.7) 5 (14.3) 8 (6.9) 27 (9.1) median score [range] 82 [23-184] 62 [23-160] 90 [26-176] 88 [21-179] 81 [21-184]

Distribution according to CURB-65 score†‡: 0-1 36 (41.9) 43 (70.5) 17 (48.6) 51 (44.0) 174 (49.3) 2 32 (37.4) 10 (16.4) 10 (28.6) 40 (33.3) 92 (27.5) 3 16 (20.9) 8 (13.1) 8 (22.9) 25 (21.5) 55 (16.5) 4 55 (64.0) 20 (32.0) 21 (60.0) 70 (59.8) 166 (55.3)

*p*Except where otherwise indicated; †Percentages are based on the number of patients for which information was available. These numbers can vary between variables; COPD = chronic obstructive pulmonary disease; ‡Use of antibiotics in the past two weeks before attending the emergency department; §Patients used two different types of antibiotics prior to hospitalisation; ¶Influenza-like illness in the past four weeks before attending the emergency department; ¶Pneumonia Severity Score (PSI), risk class: t1-III=low, IV=moderate, V=severe; ¶CURB-65 score: 0=0=mild pneumonia; 2=moderate pneumonia; 3-5=severe pneumonia; ¶¶severe pneumonia is defined as PSI risk class IV and/or CURB=score 2.

Pneumonia had less comorbidity (OR 0.3; 95% CI 0.1-0.9) compared with patients with ‘typical pneumonia’. Age ≥70 years was statistically significantly more common in the 37 patients with mixed bacterial-viral infection, compared with the 171 patients with a single infection (OR 2.5, 95%CI 1.2-5.1). Comparing patients for whom no pathogens could be identified with those for whom one or more potential pathogens were identified showed no statistically significant differences between the two groups with respect to age ≥70 years, gender, comorbidity, prior use of antibiotics, prior ILI and smoking behaviour.

**Discussion**

While *S. pneumoniae* remains the most common pathogen identified in adult CAP patients, we also detected infection with atypical bacteria in a large proportion of patients.9,12,13,16,17 Initial empirical antibiotics should be effective against *S. pneumoniae*, which is the main reason that amoxicillin is recommended as first-choice treatment in the recently revised Dutch guidelines on the management of CAP.6,14 We identified *C. burnetii* as the second most common pathogen. This is not surprising, as our study coincided in time and place with a large Q fever outbreak in the Netherlands.15-24 However, in
Spain, in a non-outbreak setting, 72/390 (18.5%) of CAP proved to be caused by C. burnetii, illustrating that the importance of C. burnetii may be underestimated in some aetiological studies. We identified M. pneumoniae as third most common pathogen. Infection with atypical bacteria, including C. burnetii and M. pneumoniae, was found in 69 (20%) of the 339 patients included in our study, mainly in younger persons (median age 59 years) with relatively low pneumonia severity scores and little comorbidity. This is in line with other studies investigating the role of atypical pathogens in the aetiology of CAP. Due to the recent changes in the Dutch guidelines on the management of CAP, atypical bacteria are no longer covered by the first-choice antibiotic treatment, because they are generally not susceptible to amoxicillin. In the initial stage of the Q fever epidemic in the Netherlands, a quarter of the Q fever patients who were initially treated with penicillins, such as amoxicillin, had a less favourable outcome than those treated with tetracyclines, such as doxycycline. In the present study, 74% of the patients with infection with atypical bacteria received penicillins, mainly amoxicillin, prior to hospitalisation.

The Dutch Q fever outbreak as well as recent outbreaks of M. pneumoniae in several European countries show that atypical bacteria are likely to remain an important cause of CAP. This underlines the importance for physicians to be alert for atypical bacteria. Especially in younger and previously healthy patients, they could consider the use of empiric antibiotics (e.g. doxycycline) covering these bacteria. British and North American guidelines on the treatment of CAP recommend initial therapy with combinations of penicillins and macrolides, or monotherapy with quinolones for patients hospitalised with CAP. Although atypical bacteria are covered by the recommended therapies, implementation of these guidelines in the Netherlands would result in a considerable increase in antibiotic use with possible adverse consequences.

A more preferable option is to improve diagnostic algorithms including typical bacteria, atypical bacteria and viruses in the first-line diagnostics of CAP, taking into account the differences in sensitivity and specificity of the various microbiological and molecular assays.

The increased use of molecular diagnostics has improved our knowledge about the aetiology of CAP. However, the exact role of viruses in the pathogenesis of pneumonia remains unclear. Rhinovirus and RSV are known to be capable of invading and replicating in the lower respiratory tract mucosa, but it is unclear whether such a virus infection is a primary cause of pneumonia or paves the path for a secondary bacterial infection. Mixed bacterial-viral infections are increasingly diagnosed in CAP patients with rates varying between 6-35%, corresponding to the 11% we found. Furthermore, our study confirmed that the commensal pathogens S. pneumoniae and H. influenzae are commonly identified in patients with mixed bacterial-viral infections. On the contrary, C. burnetii was rarely involved in these mixed infections.

Although S. pneumoniae is the most commonly detected pathogen in CAP in many studies worldwide, the proportion of other detected micro-organisms, particularly atypical pathogens, varies by region as well as over time. Since our study by chance coincided in time and place with a large Q fever outbreak, the results cannot be extrapolated directly to other years and other countries. To improve the insight in occurrence, fluctuations and seasonality of pathogens associated with CAP, continuous microbiological surveillance is therefore preferred over aetiological studies. Since flora detected in respiratory specimens are not necessarily causative pathogens, but can also indicate the presence of commensal micro-organisms or asymptomatic infections, it is important to include information on clinical presentation in microbiological surveillance.

One of the strengths of our study is the well-defined study population: various data on both patient characteristics and microbiological surveillance is therefore preferred over aetiological studies. Since flora detected in respiratory specimens are not necessarily causative pathogens, but can also indicate the presence of commensal micro-organisms or asymptomatic infections, it is important to include information on clinical presentation in microbiological surveillance.
cyst X-ray for all patients. An additional strength is the use of an extensive combination of microbiological and molecular techniques, including molecular diagnostics on both bacterial and viral pathogens. Nevertheless, no pathogens could be detected in 40% of the patients. This is in line with several recent studies on the aetiology of CAP, reporting a failure of pathogen detection for 24-44% of the patients.12,13,15,16,40-50. Because of this diagnostic deficit, which might partly be explained by the difficulty to collect paired serum samples as well as high quality sputum samples, we undoubtedly underestimated the prevalence of several pathogens. Another limitation of our study was the absence of a control group enabling the investigation of a causal link between clinical disease and detected pathogens. In a previous case-control study, we detected respiratory pathogens, mainly viruses, in ~30% of the persons without respiratory symptoms at the moment of specimen collection.11 To optimise the comparability, controls should be matched to case patients by sample collection method. However, this is impossible for sputum samples since asymptomatic persons do not produce sputum.

In conclusion, our study showed that infection with atypical bacteria is commonly found in patients hospitalised with CAP. Especially in relatively young patients without underlying medical conditions and with relatively low pneumonia severity scores, physicians should be alert for atypical bacteria, which are not covered by the current first-choice antibiotic treatment according to the Dutch guidelines on the management of CAP. Improved diagnostic algorithms including both typical bacteria, atypical bacteria and viruses in the first-line diagnostics of CAP might be helpful for the dilemma whether to use empirical amoxicillin as most effective against S. pneumoniae or doxycycline as effective against most atypical bacterial pathogens.

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**Abstract**

Malaria tropica is almost exclusively diagnosed within two months after returning from an endemic country. We present here a male patient with severe *P. falciparum* malaria diagnosed 2.5 years after returning from Burkina-Faso. We speculate that our patient was chronically infected with PF malaria for more than 2 years, with an undetectable parasite index and without symptoms. Because of waning immunity clinically overt PF malaria was able to develop. This case illustrates the importance of malaria suspicion as a cause of illness in immigrants from malaria-endemic countries. Even when these immigrants did not travel for a long time, malaria should be considered in patients with typical symptoms.

**Keywords**

*Plasmodium falciparum*, recrudescence, malaria, late-onset

**Introduction**

Malaria caused by *Plasmodium falciparum* is an important cause of morbidity and mortality in travellers returning from an endemic country. Ex-pats are at increased risk as they have waning immunity but do not usually comply with prophylaxis. In contrast to malaria caused by *P. vivax* and *P. ovale*, which can relapse years after infection due to the hypnozoite stage of these species, *P. falciparum* malaria has no hypnozoite stage and is therefore almost exclusively diagnosed within two months after returning from an endemic country.

As an exception to this rule, we present here a male patient with severe *P. falciparum* malaria diagnosed 2.5 years after returning from Burkina-Faso.

**Case Presentation**

A previously healthy 48-year-old man was admitted to the Canisius-Wilhelmina Hospital in Nijmegen, the Netherlands with a five-day history of general malaise, fever, chills, profuse transpiration and bloody diarrhoea. Originally from Burkina-Faso, he immigrated to the Netherlands eight years before presentation. The patient had not visited his homeland or any other malaria-endemic country in the previous 2.5 years. No recurrent fever episodes were noted during this period. The last time he took malaria prophylaxis was in 1997. There was no history of blood transfusions. No friends or relatives from Burkina Faso had visited him in the last two months.

Physical examination revealed a sick, icteric patient. He was alert and oriented. Body temperature was 36.8°C, blood pressure 135/85 mmHg, and pulse 90 beats/min. Oxygen saturation was 96% with a respiratory rate of 40/min. There was no rash or lymphadenopathy. Cardiac examination revealed normal heart sounds without murmurs. The lungs were clear to auscultation. There were no palpable abdominal masses, nor hepatomegaly or splenomegaly. Neurological examinations were normal. Laboratory results were as follows: C-reactive protein 149 mg/l, haemoglobin 7.8 mmol/l, thrombocytes 14 x 10^9/l, leucocytes 9.1 x 10^9/l, lactate 4.9 mmol/l, glucose 5.3 mmol/l, creatinine 184 µmol/l, bilirubin 218 µmol/l, lactate...
Berrevoets et al. Late recrudescence of *Plasmodium falciparum* malaria.

**Figure 1.** Erythrocytes with ring-shaped trophozoites and a schizont

The incubation time of PF malaria is typically less than one month; most PF infections occur in the first two months after arrival. However, cases of prolonged PF malaria have been described and diagnosed even nine years after living outside an endemic area. In contrast, in other malaria species, including *P. vivax* or *P. ovale* infection, late-onset is more common. In 62.3% of returning travellers with malaria due to *P. vivax* or *P. ovale*, infection developed more than two months after the traveller’s return.

Because most PF malaria patients develop symptoms within two months after the bite of an infected *Anopheles* mosquito, clinicians will initially not suspect the diagnosis of PF malaria, even in patients with typical symptoms. It is important to identify risk factors for the long interval between exposure in a malaria-endemic area and the clinical presentation with a febrile illness.

In *P. vivax* and *P. ovale*, hypnozoites can survive in the liver for many years and finally cause clinical malaria. PF does not have a hypnozoite form, so other causes for late-onset infections have been subject of debate. Ortenzio et al. performed a case-control study to evaluate risk factors for prolonged PF infection in immigrants. During a ten-year follow-up period, 61 (2.3%) late infections (>59 days after returning from an endemic area) occurred among 2680 diagnosed PF malaria infections. Median diagnosis delay was five months.

Four patients had clinical malaria more than three years after return from a malaria endemic area and all of them were pregnant women. Among immigrant travellers, three groups had a higher risk for prolonged PF infection: first-arrival immigrants, pregnant women and/or those taking mefloquine prophylaxis. The first two factors most likely reflect partial control of parasitaemia by acquired immunity.

Several immunological mechanisms have been suggested to explain late manifestations of PF malaria. Immunity to malaria evolves relatively slowly and is said to decrease quickly when immune adults leave malaria-endemic countries, suggesting that continued exposure to malarial antigens is essential for maintained immunity. Otherwise, immunity to malaria is temporary. This semi-immunity status does not prevent infection, but seems to protect against severe malaria and clinical symptoms. However, there is no agreement as to which components of immunity to malaria are lost without exposure, and this loss is often identified only by the fact that such people do experience symptomatic infections. Pregnant women living in malaria endemic countries are vulnerable to develop symptomatic PF malaria even when pre-existing acquired immunity was present to control low parasitaemia.

This loss of immunity is caused by a change in antigens in placenta-dwelling parasites. Primigravidae are fully susceptible to PF malaria because the antigens expressed...
on the erythrocyte surface by placental parasites are distinct from those expressed by other PF. New infection with PF is not necessary to initiate placental infection in a woman with low parasitaemia at time of pregnancy as parasites can switch between different surface antigens.\textsuperscript{13,14}

A special risk group for late-onset PF malaria are immigrants from endemic countries who travel to their country of origin to visit friends and relatives (VFR).\textsuperscript{15} They have a higher risk for infectious diseases, because they often travel for longer periods of time and do not seek pre-travel advice.\textsuperscript{9} Although the number of infections diagnosed in VFR has declined, it is important to recognise this group because of the possibility of a subclinical and delayed-onset type of PF malaria.

Although PF malaria is usually contracted in the tropics from bites of infected female \textit{Anopheles} mosquitoes, it can also be transmitted via blood transfusion, bone marrow transplants, needle-stick injuries and by the introduction of infected mosquito vectors on aircraft, e.g. suitcase or baggage malaria and airport malaria have been a cause of PF infections.\textsuperscript{16-20} When these modes of transmission have been excluded, late recrudescence is most the likely cause of disease.

In our case, we found no evidence for any of the above explanations for his PF malaria infection. There was no history of recent travelling or visiting friends from Burkina Faso, nor did our patient import tropical plants which could be a source for the \textit{Anopheles} mosquito.\textsuperscript{21} Another argument for late-onset malaria instead of indigenous malaria is the fact that the patient became ill in winter; at that time it was freezing, a time when proliferation of the vector in the Netherlands should be very difficult.

We considered whether the patient might have withheld anamnestic data on purpose. He was repeatedly asked about his travel history and visiting friends from endemic countries, but his answer remained the same and seemed to be reliable.

We speculate therefore that our patient was chronically infected with PF malaria for more than two years, with an undetectable parasite index and without symptoms. Because of waning immunity clinically overt PF malaria was able to develop. We found no underlying systemic disease which could have led to decreased immunity.

This case illustrates the importance of malaria suspicion as a cause of illness in immigrants from malaria-endemic countries. Even when these immigrants did not travel for a long time, malaria should be considered in patients with typical symptoms.

\textbf{REFERENCES}


Berrevoets et al. Late recrudescence of \textit{Plasmodium falciparum} malaria.
A 62-year-old woman reported having episodes of sudden, spontaneous painful dark discolouration of her right middle finger. Such episodes had been occurring for a few years, with a frequency of once per 4-6 months, lasting 3-5 days on average. Elsewhere, she had been given a diagnosis of 'atypical Raynaud syndrome', but later on, another physician suspected arterial embolism and recommended invasive angiography of the aortic arch and brachial arteries. She declined, and asked to be referred for a second opinion.

WHAT IS YOUR DIAGNOSIS?

See page 433 for the answer to this photo quiz.
A 56-year-old man with tongue lesions

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CASE REPORT

A 56-year-old man from Suriname who had been living in the Netherlands for the last 30 years presented in the outpatient department with a five-month history of a painless swelling of the tongue. Seven years earlier he was investigated because of pleural effusion, classified as a pleuritis of unknown origin. Testing for tuberculosis at that time was negative. There was no history of fever, weight loss or night sweats. The patient complained of a dry cough and dyspnoea on exercise. Local examination revealed an irregular nodular swelling in the middle of the tongue (figure 1). There was no lymphadenopathy and breath sounds were normal. The routine blood investigations were within normal limits except for a raised erythrocyte sedimentation rate (32 mm in the first hour). The anteroposterior chest X-ray showed consolidations in the right and left upper lobe without lymphadenopathy (figure 2).

WHAT IS YOUR DIAGNOSIS?

See page 434 for the answer to this photo quiz.
Case report

A 36-year-old male presented with a two-week history of abdominal pain and chills. His medical history was unremarkable. His vital signs were normal except for mild pyrexia of 37.6 °C. Examination of the abdomen revealed some tenderness in the mid-abdominal region. His liver enzymes were elevated with a total bilirubin 200 µmol/l (normal 0-17 µmol/l), aspartate aminotransferase 92 IU/l (normal 0-40), and gamma glutamyl transpeptidase 100 U/l (normal 0-60 U/l). Ultrasound and computed tomography (CT) scan of the abdomen revealed thrombosis of the mesenteric and portal veins. Blood cultures were positive for *Escherichia coli*, *Streptococcus millerus* and *Staphylococcus epidermidus*. Because of progressive sepsis with circulatory and respiratory failure the patient was admitted to the intensive care for intubation and resuscitation. Two days later a CT scan of the abdomen was repeated, which showed multiple hypodense lesions in the liver consistent with abscesses.

What is your diagnosis?

See page 435 for the answer to this photo quiz.
SKIN MARKS IN SURINAMESE PEOPLE

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CASE REPORT

Patient A, a 33-year-old female, with a history of human immunodeficiency virus (HIV) seropositivity with a CD4 cell count of 237 cells/mm³ and an undetectable viral load, was admitted to a Surinamese hospital after being diagnosed with a lobar pneumonia of the right upper lobe. On physical examination, she showed vesiculae on her abdomen (figure 1).

Patient B, a 39-year-old female, was admitted to a Surinamese hospital with fever. She had been feeling ill for one month, and noted temperatures up to 38°C. She had periods of headaches and pain in the neck. On physical examination, she showed no abnormalities except two spots on her forehead (figure 2). Both photographs were taken with permission of the patients.

WHAT IS YOUR DIAGNOSIS?

See page 436 for the answer to this photo quiz.
The story this patient told is compatible with Achenbach syndrome, or 'paroxysmal finger haematoma'. Her physical examination at that time, including blood pressure, arterial pulsations, finger capillary refill and Allen test, was perfectly normal. No additional tests were ordered, but we requested her to come and see us to show her finger during an episode. She did not do this, but a few months later, she sent this picture by e-mail from her vacation address in southern France (figure 1).

The history as well as the image is typical of paroxysmal finger haematoma. This is a benign, idiopathic disorder, which has been described in the medical literature since the early 1960s. Its prevalence is unknown, but appears to be highest in middle-aged women. Characteristically, there is bruising of the volar side of a finger, which occurs either spontaneously or after minor trauma. Its pathogenesis is unknown.

Paroxysmal finger haematoma is rarely recognised immediately by clinicians, and unnecessary additional tests, including invasive procedures such as angiograph, may be ordered.

**REFERENCES**


Histopathology of the swelling disclosed a granulomatous reaction with necrosis and the Ziehl Neelsen stain for acid-fast bacilli was positive. Microscopic examination of the sputum also revealed acid-fast bacilli and a polymerase chain reaction of the sample was positive for Mycobacterium tuberculosis complex. The Mantoux test performed measured 20 mm after 72 hours. We concluded that our patient suffered a lingual manifestation of tuberculosis secondary to an active pulmonary tuberculosis. Antitubercular treatment for a period of six months was started.

Tuberculosis of the tongue, or lingual tuberculosis, is an uncommon presentation of M. tuberculosis infection. The oral cavity accounts for 0.2 to 1.5% of all the cases of extrapulmonary tuberculosis. Most cases are secondary to pulmonary tuberculosis and rarely primary in origin. In patients with tuberculosis of the oral cavity, pain and odynophagia (painful swallowing) are the most commonly reported local symptoms (both 15%). Less frequently dysphonia, burning sensation, reflux, excessive salivation, halitosis, and intra-oral bleeding are present. Tuberculosis of the tongue usually presents as a chronic non-healing mucosal ulceration but may occur as a swelling, charge with or without fistulae, nodules, fissures, or granulomatous plaques.

Other differential diagnoses include traumatic ulcers, aphthous ulcers, actinomycosis, histoplasmosis, syphilitic ulcer, neoplasms and Wegener’s granuloma. Histopathological analysis is essential to confirm the diagnosis, by finding necrotising granulomas and demonstrating acid-fast bacilli or Mycobacterium species. Although rare, tuberculosis should be included in the differential diagnosis in patients presenting with a mucosal lesions in the oral cavity.

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The patient was diagnosed with a pylephlebitis, portal-mesenteric thrombosis, and multiple liver abscesses. Pylephlebitis, or infective suppurative thrombosis of the portal vein, is a serious condition with significant morbidity and mortality, which can complicate intra-abdominal sepsis of any aetiology. Pylephlebitis is caused by a thrombophlebitis of small veins draining an area of infection. Extension of the thrombophlebitis into larger veins leads to septic thrombophlebitis of the portal vein resulting in septic emboli to the liver. Since an abdominal infection was very likely in this case, all CT scans were revised. In retrospect these also revealed appendiceal wall thickening with periappendiceal fat straining consistent with acute appendicitis. In 1898, Dieulafoy described the association between appendicitis and liver abscesses as le foie appendiculaire. Pyogenic hepatic abscesses are rare with less than 10% of cases caused by appendicitis. Pylephlebitis can complicate any intra-abdominal infection that occurs in the region drained by the portal venous system, such as diverticulitis, inflammatory bowel disease, pancreatitis and cholangitis.

The patient was treated with broad-spectrum antibiotics (ceftriaxone, metronidazole and vancomycin) and anticoagulation. Yet he showed no clinical improvement, but progression of the liver abscesses and an abdominal compartment syndrome due to major abdominal bleeding. An appendectomy was performed and five litres of old blood were removed. Postoperatively, the patient recovered well and was treated with antibiotics for another six weeks. Anticoagulation was continued for another three months after the laparotomy. No hypercoagulable status was found.

The conservative management of complicated appendicitis is associated with a decrease in the complication and reoperation rate compared with acute appendectomy and it has a similar duration of hospital stay. But as this case shows, some patients whose symptoms fail to resolve still need surgical intervention. There are neither prospective randomised controlled studies nor consensus on the use of anticoagulation in pylephlebitis. The rationale for anticoagulation in acute pylephlebitis is the prevention of thrombus extension and its sequelae. If there is mesenteric vein involvement (as in this case), patients may benefit from anticoagulation, since the risk of bowel ischaemia is higher.

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D I A G N O S I S

Both patients suffered from burn wounds due to application of locally produced heated Surinamese palm rum containing 90% alcohol. Patient A was immunocompromised due to HIV infection, but showed an undetectable viral load during therapy. Her skin marks were differentiated from herpes zoster because they crossed the mid-line of her upper abdomen. In order to relieve the feeling of dyspnoea, her grandmother had advised her to apply heated palm rum on her chest which resulted in a second-degree burn wound. Patient B suffered from feelings of fever and headache. She was told by her mother to relieve her complaints by applying clothes dragged in heated palm rum on her head. After taking the clothes away, she noticed the skin marks which were diagnosed as first-degree burn wounds.

Application of (heated) fluids with a high percentage alcohol for a wide spectrum of symptoms is one of many traditional concepts of medicine in Suriname, locally known as ‘oso dresi’ (home remedies). It is important to realise that not only Surinamese people in Suriname, but also the Surinamese immigrants and descendants from Suriname living in other countries than Suriname may stick to their traditional health beliefs and practices, which may induce side effects not commonly encountered in the country of immigration.1 Of Surinamese people living in Suriname, 86% reported to have used traditional medicine at some point in their life and 66% during the last year, while for Surinamese people living in the Netherlands this was 77% and 66%, respectively.1,2 Traditional medicine is used for health promotion, disease prevention and cure mainly of colds, headache and intestinal problems. Belief in and familiarity with its healing properties and religious knowledge were reported to predict its use, while gender, income, employment and education were not,2 arguing that traditional medicine is a deeply rooted cultural preference. Therefore, since cultures intermix easily nowadays due to migration, it is important for healthcare workers to know what (side) effects of traditional medicine may have to be considered in the differential diagnosis.3

These cases illustrate the importance of (side) effects of traditional medicine in the differential diagnosis of symptoms and signs, for example skin manifestations. Surinamese and descendants from Suriname living abroad may use traditional medicine as a first method in order to relieve symptoms.

R E F E R E N C E S

Keeping venomous snakes in the Netherlands: a harmless hobby or a public health threat?

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ABSTRACT

Objective: To describe the incidence of venomous snakebites and the hospital treatment thereof (if any) amongst private individuals who keep venomous snakes as a hobby.

Structure: Descriptive study.

Method: Private keepers of venomous snakes were invited via the social media Facebook, Hyves, Twitter, Google Plus, LinkedIn and two large discussion forums to fill in an online questionnaire on a purely voluntary and anonymous basis.

Results: In the period from 1 September 2012 to 31 December 2012, 86 questionnaires were completed by individuals who keep venomous snakes as a hobby. One-third of the venomous snake keepers stated that they had at some point been bitten by a venomous snake. Out of those, two-thirds needed hospital treatment and one-third of those bitten required at least one, sometimes more, doses of antiserum. The chances of being bitten increased the more venomous snakes a person kept. An inventory of the collections of venomous snakes being kept further revealed that no antiserum exists for 16 of the species, including for the most commonly held venomous snake, the coral cobra.

Conclusion: Keeping venomous snakes as a hobby is not without danger. Although in the majority of snakebite cases no antiserum had to be administered, there is nevertheless a significant risk of morbidity and sequelae. Preventing snakebites in the first place remains the most important safety measure since there are no antiserums available for a substantial number of venomous snakes.

INTRODUCTION

Keeping reptiles is a popular hobby in the Netherlands. In total an estimated 35,000 households are believed to house 250,000 reptiles between them. The keeping of dangerous and venomous reptiles including venomous snakes is not banned in the Netherlands. The relevant (local law) regulations (‘APV’) do not contain any specific restrictions on the keeping of such snakes. Some municipalities such as Utrecht have introduced certain requirements but these usually amount to no more than a requirement to notify. Currently, no municipalities require residents to have a license for keeping dangerous animals and no attempt is being made to control the large number of poisonous animals being held by hobbyists by means of environmental licences. Regardless of which rules are appropriate, it appears that keeping venomous snakes as a hobby takes place largely in anonymity. Bites by exotic venomous snakes are, however, widely reported in the media, almost exclusively in a very one-sided sensationalist manner.2

The Havenziekenhuis in Rotterdam treats on average one to three people annually who have been bitten by (exotic) venomous snakes. The protocol for the care of and emergency help for victims of venomous snakebites was previously published3 and forms the basis for the national exotic (venomous) snake protocol.4 It is striking that the snakebites treated in our hospital are actually always bites by exotic venomous snakes and never bites by the adder (Vipera berus), the only true indigenous venomous snake in the Netherlands. However, recent research has shown that in the vicinity of Poortugaal in South Holland there is a small population of the Aspis adder (Vipera aspis), which is not native but was probably consciously or unconsciously
introduced by someone.' These snakes could be a potential danger to hikers in this area.

It is likely that the number of exotic venomous snakebites treated in hospital is just the tip of the iceberg because these are the only ones which come onto our radar. The full picture of what type and how many incidents of venomous snakebites are experienced by hobbyists is unclear. In order to gain more insight into the prevalence of snakebites amongst keepers of venomous snakes and into the scale and diversity of collections of venomous snakes amongst hobbyists, we have conducted a survey amongst keepers of such snakes via the Internet. The results of this study are given below.

**Patients and Methods**

The Working Group 'Venomous Snakes Havenziekenhuis' has conducted a web-based survey over the Internet of people who keep venomous snakes as a hobby. The target group was invited through the social media Facebook, Hyves, Twitter, GooglePlus, LinkedIn and the two largest relevant discussion groups 'The Snakes Forum' and the forum of 'the Target Group Venomous Snakes Lacerta' to answer the following questions on a purely voluntary and anonymous basis:

1. In which province do you keep your snakes?
2. What type(s) of venomous snakes do you keep?
3. How many venomous snakes do you have (approximately)?
4. How long have you been keeping venomous snakes for?
5. Have you ever been bitten by one of your venomous snakes?
6. How many times was hospital treatment necessary for your snakebite(s)?
7. If you have had a venomous snakebite, have you ever been treated with antivenom?

To ensure (as far as possible) the uniformity of responses, the maximum possible number of closed questions were used with a limited number of choices.

**Results**

In the period from 1 September 2012 to 31 December 2012, the questionnaire was completed by 86 keepers of venomous snakes. As shown in figure 1, the largest number of these keepers were living in the provinces of South Holland (n = 21, 24.4%), Noord-Brabant (n = 15, 17.4%) and Gelderland (n = 13, 15.1%). Thirty-two (37.2%) of the respondents had 1-5 venomous snakes, while 18 people (20.9%) had more than 20 snakes. Forty-five (52.3%) of the respondents had been keeping snakes as a hobby for less than five years, whilst 12 of them (14.0%) had been doing so for over 15 years. Fifty-four (64.3%) respondents indicated that they had never been bitten by their venomous snake, 21 (25.0%) of them reported having been bitten once, seven (8.3%) had been bitten between 2-4 times, one (1.2%) respondent had been bitten between 5-9 times and one (1.2%) respondent actually reported having been bitten ≥10 times by a venomous snake. Nineteen (23.4%) respondents reported having had to go to hospital for treatment one or more times. Twelve (14.1%) had been to hospital just once for treatment, five (5.9%) needed to go to hospital between 2-4 times, one (1.2%) respondent had been between 5-9 times and one (1.2%) other person had been to a hospital for treatment ≥10 times. As part of their treatment 11 (13.2%) individuals had had an antiserum administered in a hospital. One person (1.2%) was given antiserum on several occasions. In figure 2, the flowchart shows the number of venomous snakebites as well as any treatment in a hospital and the administration of antiserum amongst the 86 respondents.

**Risk factors for snakebites**

A significant trend was observed between on the one hand the number of snakes being kept and the number of snakebites reported (p value = 0.0013) and on the other hand between the number of snakebites and the number of years that the hobby was exercised (p value = 0.0139). There was also a significant trend between the number of
Figure 2. Flowchart illustrating the responses of the 86 individuals who took part in the Internet survey concerning the incidents of venomous snakebites, the number requiring hospital treatment and those resulting in the administration of antiserum.

86 snake keepers

Ever been bitten?

Yes → 30 persons

No → 56 persons

Number of bite incidents?

Single incident

Multiple incidents

21 persons

In-hospital treatment?

No → 10 persons

Yes → 11 persons

Antiserum?

No → 6 persons

Yes → 5 persons

5 persons single episode with administration of antiserum

9 persons

In-hospital treatment?

Yes → 8 persons

No → 1 person

Antiserum?

No → 2 persons

Yes → 6 persons

1 person multiple episodes with administration of antiserum
years that the snakes were kept as a hobby and the number of snakes (p value <0.0001).

**Extent and diversity of venomous snake collections**

Table 1 lists the top five species of venomous snakes that are being kept, broken down by family. From the pit viper or pit adder (*Crotalinae*) family there are 43 species (with 31 subspecies) with the neotropical rattlesnake (*Crotalus durissus*) the most popular (15 times). From the family of coral snakes (*Elapidae*) there were 34 species with 11 subspecies. The coral cobra (*Aspidelaps lubricus*) is the most commonly kept within this family (30 times). Amongst the family of true vipers (*Viperinae*), 24 species with nine subspecies were being held and the sand viper (*Vipera ammodytus*) was the most popular adder (16 times). It also appears from the survey that in addition to the above there are four types of colubrids (*Colubridae*) being kept.

Out of all the snake species identified by the survey, it is worth noting that no antiserum exists for 16 species, and this includes the most popular species, the coral cobra.

**Discussion**

Because most people who keep venomous snakes as a hobby do so in private, little is known about the number of incidents involving exotic venomous snakes, or about the exact size and diversity of collections of venomous snakes. Since its inception in 2008, the National Serum Depot, as part of the National Institute for Public Health and the Environment and the National Poisons Information Centre (NVIC), has been the institute that coordinates the distribution of antiserum. A recent publication revealed that in the period between 2008 and December 2011, the NVIC was on average consulted five times per year over a bite from an exotic venomous snake, whilst during the same period an antidote was only issued five times in total, and was actually administered on just two occasions. No fatalities caused by bites from exotic venomous snakes have been recorded in the Netherlands. These observations suggest that a significant proportion of such bites trigger only mild reactions, that the administration of antiserum is not always necessary and that it is quite possible that many of the bites are so called ‘dry’ or defensive bites. Our survey gives a similar picture. On average, one in three keepers of snakes confirmed having been bitten by exotic venomous snakes whilst keeping them as a hobby. Of the 30 people bitten by these snakes, 11 of them (36.7%) did not require any hospital treatment. Of the remaining 19 people who reported having been bitten, 11 had been given an antiserum at least once in their lives. Trend analysis shows a clear relationship between the occurrence of snakebites and the number of snakes being kept (or the size of the venomous snake collection) but also with the number of years that a person has been keeping snakes as a hobby (or the number of times he/she has been exposed to venomous snakes). Unfortunately, the survey did not ask about the circumstances under which the bites occurred, such as whether this was during the cleaning of the terrarium, while changing the water, or whilst handling the snakes.

The survey has provided detailed information about the extent and diversity of venomous snake collections belonging to the 86 respondents. There is no antiserum against 16 of the species of venomous snakes being kept. This means that even more emphasis needs to be put on the prevention of venomous snakebites. Further research revealing the circumstances in which snakebites are occurring would be valuable when preparing advice for persons keeping venomous snakes about what preventative measures they should take.

**Table 1.** Listing of the top five species (broken down by family) of venomous snakes that are being kept by the 86 internet respondents

<table>
<thead>
<tr>
<th>Family name</th>
<th>Number of times kept</th>
<th>Existing antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Pitvipers (<em>Crotalinae</em>)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neotropical rattlesnake (<em>Crotalus durissus</em>)</td>
<td>15 (4 subspecies)</td>
<td>Yes</td>
</tr>
<tr>
<td>Western Diamond Back rattlesnake (<em>Crotalus atrox</em>)</td>
<td>12</td>
<td>Yes</td>
</tr>
<tr>
<td>Massasauga (<em>Sistrurus catenatus</em>)</td>
<td>28 (3 subspecies)</td>
<td>No</td>
</tr>
<tr>
<td>Copperhead (<em>Agkistrodon contortrix</em>)</td>
<td>9 (3 subspecies)</td>
<td>Yes</td>
</tr>
<tr>
<td>White-lipped Bamboo viper (<em>Trimeresurus albolabris</em>)</td>
<td>8</td>
<td>Yes</td>
</tr>
<tr>
<td>II. Elapid snakes (<em>Elapidae</em>)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coral Cobra (<em>Aspidelaps lubricus</em>)</td>
<td>28 (3 subspecies)</td>
<td>No</td>
</tr>
<tr>
<td>Snouted Cobra (<em>Naja annulifera</em>)</td>
<td>12</td>
<td>Yes</td>
</tr>
<tr>
<td>Monocle Cobra (<em>Naja kaouthia</em>)</td>
<td>11</td>
<td>Yes</td>
</tr>
<tr>
<td>Indo-Chinese Spitting Cobra (<em>Naja atra</em>)</td>
<td>3</td>
<td>Yes</td>
</tr>
<tr>
<td>Cape Cobra (<em>Naja nivea</em>)</td>
<td>8</td>
<td>Yes</td>
</tr>
<tr>
<td>III. Vipers (<em>Viperinae</em>)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-nosed viper (<em>Vipera ammodytus</em>)</td>
<td>16 (2 subspecies)</td>
<td>Yes</td>
</tr>
<tr>
<td>Puff viper (<em>Bitis arietans</em>)</td>
<td>9</td>
<td>Yes</td>
</tr>
<tr>
<td>Sahara Horned viper (<em>Cerastes cerastes</em>)</td>
<td>6</td>
<td>Yes</td>
</tr>
<tr>
<td>Gabon viper (<em>Bitis gabonica</em>)</td>
<td>5 (2 subspecies)</td>
<td>Yes</td>
</tr>
<tr>
<td>Asp viper (<em>Vipera aspis</em>)</td>
<td>5</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Further information is important not only for individuals keeping snakes but also for the emergency services, such as those at the Havenziekenhuis, which provide specialised help in the case of an (exotic) venomous snakebite. It would also assist with the proper alignment of the stock and diversity of antiserums against bites from venomous snakes. In order for such an initiative to succeed, help from individuals keeping snakes and an open dialogue between them and the emergency services is essential.

LIMITATIONS

The incidence of snakebites identified in the survey must be considered in a specific but probably also limited context, due to inherent recall and response biases. Because many snake owners exercise the hobby in anonymity, the questionnaire was written in such a way as to ensure that it was not traceable to any individual. And in order to guarantee the anonymity of the persons keeping venomous snakes we specifically did not ask which year an individual was bitten or, if this was the case, in which year they received in-hospital treatment, but only whether the respondents had ever been bitten during the time that they kept venomous snakes as a hobby and whether or not they required hospital treatment. In addition, the question remains whether the persons who took part in the study are representative of all individuals who keep exotic venomous snakes as a hobby, the exact number of whom in the Netherlands is unknown.

CONCLUSION

Keeping venomous snakes as a hobby is not without danger. The chances of being bitten increase the more snakes are kept and the longer they are kept for. Although there have been no fatalities recorded in the Netherlands as a result of bites of exotic venomous snakebites, a considerable number of such bites require the administration of an antiserum. Because there are a significant number of species of venomous snakes for which no antiserum exists, the prevention of bites by venomous snakes remains the most important safety measure.

ACKNOWLEDGEMENTS

We acknowledge the trust put in us by all respondents to the questionnaire.

REFERENCES

Dear Editor,

We want to draw attention to the important role of the parathyroid glands in maintaining normocalcaemia. A 50-year-old woman with known hypoparathyroidism secondary to Riedel’s thyroiditis initially presented with severe hypercalcaemia and acute renal failure luxated by a gastroenteritis (table 1). Her medications dihydrotachysterol (DHT; 0.4 mg/day), calcium carbonate (3000 mg/day) and hydrochlorothiazide were stopped and she was treated with normal saline and a single dose of 60 mg pamidronate. After recovery of the hypercalcaemia she was discharged on her previous medication (figure 1).

Five days later she presented again, now with severe hypocalcaemia necessitating treatment with intravenous calcium glubionate (2160 mg/day) and oral calcium carbonate (4500 mg/day) for several weeks, and a doubled vitamin D dose (table 1, figure 1). Only after 21 days the intravenous calcium supplementation was completely tapered. Twenty-three days after admission, she was discharged on oral calcium carbonate (6000 mg/day) and alfalcaldol (0.50 µg/day) (figure 1).

A clustering of features contributed to the bidirectional extreme calcium plasma concentrations in this patient. In normal conditions plasma calcium is tightly regulated by a dynamic hormonal system that controls its transport in the gut, kidney, and bone. Key players are the parathyroid hormone (PTH) and active vitamin D (1,25(OH)2D). Whereas normally any tendency towards hypocalcaemia or hypercalcaemia will result in a rapid increase or decrease in PTH secretion from the parathyroid glands, this response was obviously lacking in our patient. In addition, she was treated with calcium carbonate and DHT. The latter is a half synthetic vitamin D analogue that does not require renal hydroxylation. DHT is deposited in fat, liver, skin, muscle and bone. After withdrawal it may take up to nine weeks for the physiological effects to completely resolve. Thus, while gastroenteritis was the initial trigger for volume depletion, hypercalcaemia and renal insufficiency, the cascade was fuelled by continued use of calcium carbonate, DHT and hydrochlorothiazide. In this situation, intoxication with DHT is likely to occur with concomitant inappropriate ongoing intestinal calcium absorption.

Administration of pamidronate was the main trigger in the development of the severe hypocalcaemia. This synthetic analogue of pyrophosphate – a natural regulator of bone metabolism found abundantly in bone matrix – has several physiological effects resulting in a net decreased osteoclast activity. As a result the drug is very effective in correcting hypercalcaemia, especially in case of vitamin D intoxication, which is characterised by increased bone resorption. The appropriate pamidronate dose depends on patient characteristics, including the risk of hypocalcaemia, which is increased in the presence of hypoparathyroidism. A few case reports indicate low-dose pamidronate (15-20 mg) to be safe in hypoparathyroidism with DHT intoxication. Our patient received a higher dose (60 mg) instead. Although the plasma half-life of pamidronate is short, the inhibiting effect on osteoclasts persists for many weeks. Administration of APD in the presence of renal failure can result in prolonged and stronger inhibitory effects on osteoclastic activity, with a concomitant increased risk of prolonged hypocalcaemia. The long half-life of DHT, the vitamin D analogue used in our patient, is an unfavourable characteristic in the clinical situation in which immediate vitamin D effects are warranted. When the patient presented with hypocalcaemia, we therefore decided to replace the DHT by the short-acting active vitamin D analogue alfalcaldol.
In conclusion, this case illustrates the importance of functioning parathyroid glands for calcium homeostasis. In hypoparathyroid patients, bisphosphonates should be used cautiously as they can result in severe life-threatening and prolonged hypocalcaemia.

**REFERENCES**

The challenge of multidisciplinary research: improving diabetic pregnancy together

P.H.L.M. Geelhoed-Duijvestijn, M. Diamant, B.H.R. Wolffenbuttel on behalf of the Diabetes Core Group of the Dutch Association of Internists, the RT-CGM expert group of the Dutch Diabetes Federation and the board of BIDON; *corresponding author: e-mail: p.geelhoed@mchaaglanden.nl

Voormolen et al. describe the challenges for multidisciplinary research in diabetic pregnancy, referring to the reluctance of many specialists to participate in the Dutch national GlucoMOMS study. The arguments provided by the authors make it seem as if Dutch endocrinologists oppose the principle of evidence-based medicine. Instead of providing the real, i.e. scientific and potentially ethical, reasons for the relatively low national enthusiasm of referring diabetes type 1 (DM1) patients for inclusion in the GlucoMOMS trial, they state that 'doctors prefer offering their patients (un-evaluated) treatment options to offering nothing or even worse: the unpopular truth of we don’t know what’s the best thing to do'.

The GlucoMOMS study compares masked continuous glucose monitoring (CGM) during one week per month in type 1 (DM1), type 2 (DM2) and insulin-requiring gestational diabetes versus self-measured blood glucose (SMBG) monitoring. For adult (non-pregnant) DM1 patients, ample evidence exists showing that the use of real-time (RT)-CGM is better than frequent SMBG only when the device is used more than 60-75% of the time. A study which intermittently (25% of the time) applies masked CGM in pregnant DM1 patients cannot be regarded as the evaluation of a major step forward, and will not by any means contribute to gathering adequate evidence for the use of state-of-the-art RT-CGM in pregnancy. Indeed, after the GlucoMOMS study was granted, many endocrinologists suggested that the researchers update the study protocol and add a prolonged RT-CGM intervention in pregnant DM1, but to no avail.

Improving pregnancy outcomes for DM1 requires a near-normal preconceptional HbA1c and maintaining near-normoglycaemia during pregnancy and delivery. However, it takes much effort to reach this goal without frequent severe and non-severe hypoglycaemias. Currently, RT-CGM-guided pump therapy is the best technical option in non-pregnant DM1 patients. One does not have to be 'a believer' to hypothesise that this may also pertain to pregnant DM1 patients.

For adequate self-management, i.e. the mainstay of diabetes treatment already preconceptionally, masked CGM can be used as an educational tool if the treatment goal is not achieved by conventional methods, including frequent SMBG and diary use. Pregnancy, however, especially in the first 16 weeks, is associated with blood glucose fluctuations that are difficult to control and can lead to severe hypoglycaemia. Pregnant DM1 women, by feeling the responsibility of adequate glucose control for pregnancy outcome, may become insecure and vulnerable. The RT-CGM device, which helps them to stay in control and warns them in case of hyperglycaemia or hypoglycaemia, is an important supportive tool.

To further inform Voormolen et al., already in 2010 the expert group of the Dutch Diabetes Federation strongly advised the Healthcare Insurance Board (CVZ) only to reimburse RT-CGM for several indications awaiting the results of national data collection on outcome. Therefore, within a collaborative national initiative, also supported by The Netherlands Organisation for Health Research and Development (ZonMw), together with the Dutch Diabetes Patient Organisation, endocrinologists are currently collecting data in all patients using RT-CGM with many eligible centres (>50) already participating (www.stichtingbidon.nl). We agree with the authors’ remark stating that, ‘…… millions if not billions of euros are spent on ineffective and therefore by definition useless treatments’, and we are assured that they will concur with the fact that even greater budgets are spent on poorly designed and therefore a priori non-conclusive studies that advance neither clinical science nor care.

REFERENCES


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