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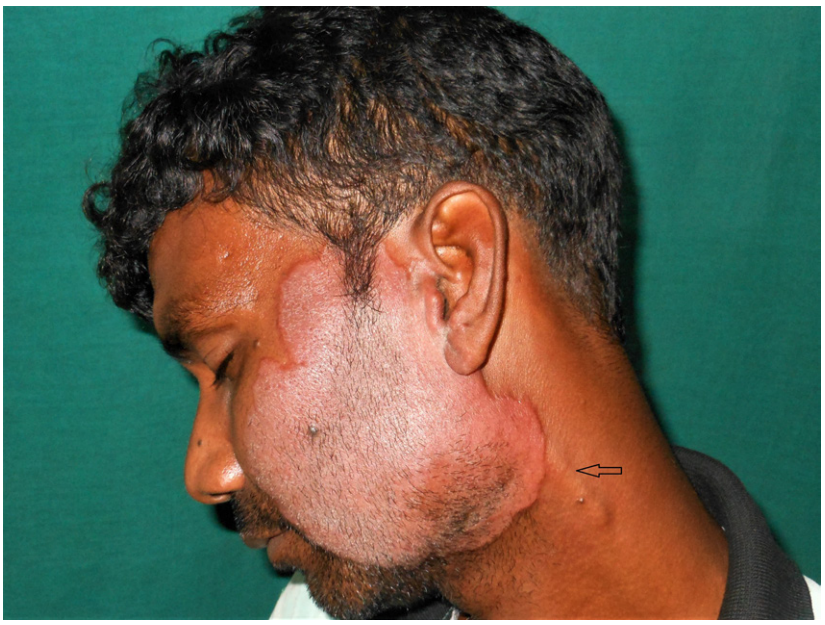

Red-coloured rash over the face. What is your diagnosis?

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MacChain

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EDITORIAL

Blood pressure variability and mortality

P.L.A. van Daele

Increased blood pressure is an important risk factor for mortality, but blood pressure varies. Ambulatory blood pressure monitoring overcomes this problem with blood pressure variability, and therefore has the advantage of detecting 'white coat' and labile hypertension. It thereby appears to be a stronger predictor of all-cause and cardiovascular mortality compared to blood pressure measured in the doctor's office.[1] However, ambulatory blood pressure measurement is inconvenient and sometimes even painful for patients, and it is much easier to just measure blood pressure in our outpatient clinic.

Oscillation of blood pressure within a 24-hour period and over prolonged periods of time has been shown to be the result of complex interactions between extrinsic environmental and behavioural factors and intrinsic cardiovascular regulatory mechanisms.[2]

The extent to which blood pressure varies over time has been recognised as a potential risk factor in its own right, as it is a predictor of stroke, renal damage, coronary events, and mortality in high risk patients independent of mean systolic blood pressure.[3-6]

Post-hoc analyses of large intervention trials in hypertension show that within-patient visit-to-visit blood pressure variation is associated with increased cardiovascular morbidity and mortality. This has prompted discussion on whether antihypertensive treatment should be targeted not only towards reducing mean blood pressure levels, but also towards stabilising blood pressure variation with the aim of achieving consistent blood pressure control over time, thereby favouring cardiovascular protection.[2]

It is still not completely understood why oscillation in blood pressure is a risk factor for cardiovascular disease, although some potential mechanisms have been identified. In this respect, Zhou et al. found that greater very short- to midterm blood pressure variation is associated with greater aortic stiffness and maladaptive carotid arterial remodelling, but not with carotid stiffness, which may explain the increased blood pressure variation-associated cardiovascular disease risk.[7]

In the current issue of the journal, Papaioannou et al. examined whether variation in blood pressure, measured three times during a single visit with an interval of five minutes, was associated with an increased risk of mortality. They saw a gradual decrease of both systolic and diastolic pressure. How bigger the difference (or variation), how higher the mortality. A gradual drop in blood pressure during consultation as risk factor for morbidity has been recognised earlier. It most likely represents a "white coat effect" which, in other studies, has been shown to be an independent risk factor for both cardiovascular and overall mortality.[8]

Nevertheless, in-office repeated blood pressure measurement can be informative regarding mortality risk. Another reason to limit the number of online consultations.

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REVIEW

Diagnostic and therapeutic strategies for porphyrias

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ABSTRACT

Porphyrias are rare metabolic disorders. Lack of awareness and knowledge about the clinical features of porphyrias results in diagnostic and therapeutic delays for many patients. Delays in diagnosing and treating porphyrias can result in severe, progressive morbidity (and mortality) and psychological distress for patients.

This review discusses the pathophysiology, diagnosis, treatment, and follow-up of the most prevalent porphyrias: acute intermittent porphyria, porphyria cutanea tarda, and erythropoietic protoporphyria.

KEYWORDS

Acute intermittent porphyria, diagnosis, erythropoietic porphyria, porphyria cutanea tarda, porphyrias

INTRODUCTION

In the majority of patients, porphyria is caused by an inherited deficiency of an enzyme of haem biosynthesis and can be classified as acute or cutaneous or a combination of both. Acute porphyrias are characterised by attacks of acute abdominal pain, often accompanied by neurological symptoms. Hypertension, chronic kidney disease, and hepatocellular carcinoma are major long-term complications of the acute porphyrias. Cutaneous porphyrias are characterised by severe pain or blistering of the light-exposed areas of the skin.

Attacks in acute porphyrias only occur in a small percentage of gene carriers (< 10%) and are often precipitated by non-inherited factors, such as the ingestion of certain drugs, weight loss, or hormonal changes. Undiagnosed acute porphyria can lead to continuance of the precipitating factors and inadequate treatment, and result in progressive neurological problems including paralysis, seizures, and coma.[1] Once a diagnosis has been made, the risk of attacks may be lowered by reducing exposure to precipitating factors; the severity of attacks may also be diminished by timely treatment.

Cutaneous porphyrias are rarely life-threatening, but can cause severe social isolation and a low quality-of-life due to avoidance of light exposure to prevent pain, or to reduce fragility or disfigurement of the skin.[2,3]

The aim of this review is to increase awareness of porphyrias and to prevent diagnostic and therapeutic delay. We provide basic information on porphyrias and practical flow charts for diagnosis and treatment to be used by health care professionals (e.g., general practitioners, internists, gastroenterologists, surgeons, neurologists, psychiatrists, and dermatologists).

Acute porphyrias

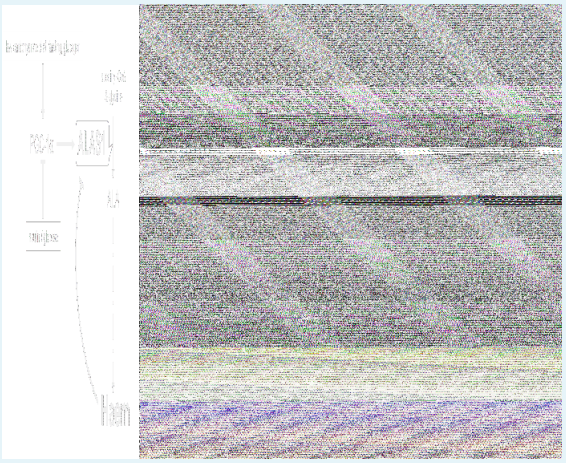
Acute porphyrias are characterised by acute attacks of severe pain, most frequently abdominal pain, sometimes only in the back or upper legs. This pain can be accompanied or preceded by symptoms such as anxiety, depression, irritability, and mood changes and followed by progressive neuropathy.[4-8] There are four different types of porphyrias that can present with acute porphyric attacks: acute intermittent porphyria (AIP), variegate porphyria (VP), hereditary coproporphyria (HCP), and delta-aminolaevulinic acid dehydratase deficiency (ALAD).[9] AIP is by far the most prevalent acute porphyria in Europe, whereas ALAD is extremely rare, with eight cases reported worldwide.[10] VP and HCP are acute porphyrias that can also present with cutaneous blistering in sunlight-exposed skin. The four different types of acute porphyria are all caused by pathogenic variants in autosomal genes, resulting in reduced activity of one of the enzymes in the haem biosynthesis pathway (table 1). Although the affected genes and enzymes differ per type of acute porphyria, the pathophysiology of these attacks is similar in all types of acute porphyria.

Table 1. *Characteristics of the four types of acute porphyria; all can present with attacks of abdominal pain.*

Name	Age of onset	Cutaneous symptoms	Enzyme	Gene (locus)	Prevalence (cases per million)*
AIP	> puberty†	No	HMBS	HMBS (11q23.3)	5.4
VP	> puberty	Blistering+	PPOX	PPOX (1q23.3)	3.2
HCP	> puberty	Blistering+	CPOX	CPOX (3q11.2)	0.2
ADP	Childhood	No	ALAD	ALAD (9q32)	< 0.0001

* Incidence reported in European countries[9]
† There are isolated case reports of patients who are compound heterozygous for mutations in the autosomal dominant acute porphyria genes, and who present with severe neurological symptoms before puberty.
+ VP and HCP can present as attacks of abdominal pain, or attacks of abdominal pain accompanied with cutaneous symptoms, or as solely cutaneous symptoms. Blisters occur only in skin exposed to (sun) light.
AIP = acute intermittent porphyria; ADP = delta-aminolaevulinic acid dehydratase deficiency porphyria; ALAD = delta-aminolaevulinic acid-dehydratase, CPOX = coproporphyrin oxidase; HCP = hereditary coproporphyria; HMBS = hydroxymethylbilane synthase; PPOX = protoporphyrin oxidase, VP = variegate porphyria

Figure 1. *Schematic representation of the basic regulation of haem biosynthesis pathway, adapted from Puy, et al.[10]*
In the first step of the hepatic haem biosynthesis pathway, succinyl-CoA and glycine are converted to delta-aminolaevulinic acid (ALA) by ALAS1, the rate-limiting enzyme of the pathway. Haem suppresses ALAS1 expression and activity at the transcriptional, translational, and post-translational levels. As haemoproteins are used for gluconeogenesis, ALAS1 is upregulated via PGC-1α to stimulate haem production during fasting (and low carbohydrate diets). This induction of ALAS1 activity induces an increase in ALA and an acute porphyria attack in HMBS gene mutation carriers with a subclinical enzyme deficiency.
Arrows indicate a stimulating effect. Capped arrows indicate an inhibiting effect. The dotted lines between ALA and haem represent the other steps in the pathway.



ALAS1 = delta-aminolaevulinic acid-synthase 1; HMBS = hydroxymethylbilane synthase; PGC-1α = peroxisome proliferator-activated receptor-gamma coactivator-1 alpha

Other than inherited deficiencies in the haem synthetic pathway, there are other rare causes of acute porphyric attacks, such as lead intoxication and tyrosinemia; lead and succinyl acetone, respectively inhibit ALAS1. The first step to diagnosing an acute attack is the same in all causes of acute porphyria. In this review, AIP will be discussed in detail.

Pathophysiology of acute attacks

Porphyria symptoms result from the accumulation of intermediates of haem biosynthesis. Acute porphyric attacks are related to an increase in ALA levels[11] and ALA accumulation seems to be the main culprit in acute porphyric attacks. The exact mechanism whereby ALA causes the neurological symptoms is unknown. Acute porphyric attacks are seen in conditions with isolated increases in ALA without a rise in porphobilinogen (PBG), including tyrosinemia, lead poisoning, and ALAD deficiency. Attacks can be provoked by precipitating factors that induce ALAS1 and ALA levels, either directly or via haem depletion. Well-described provoking factors are cytochrome P450-inducing drugs, infections, fasting, and alcohol use.[12-16] Haem production by hepatocytes is regulated differently to that in erythroid cells. In the liver, haem as an end-product, regulates haem production (figure 1) by suppressing ALAS1 expression at the transcriptional, translational, and post-translational levels.[17,18]

In patients with hereditary coproporphyria and variegate porphyria, provoking factors can result in an acute porphyric attack due to increased ALA, but they can also present with cutaneous symptoms related to increased coproporphyrin and protoporprhyrinogen levels, respectively.[9]

Symptoms, demographics, and complications of acute porphyrias

In all forms of acute porphyria, an acute attack may start with changes in behaviour and restlessness. During an

attack, nearly all patients have severe abdominal pain. This can be accompanied by anxiety, vomiting, constipation, diarrhoea, tachycardia, hypertension, hyponatraemia, red urine, muscle weakness, and neurological symptoms such as paraesthesia, paralysis, and seizures. Severe motor neuropathy can lead to respiratory failure and posterior reversible encephalopathy syndrome, with headaches, visual symptoms, and seizures, which have been observed during attacks. If left untreated, severe neurological damage and even death, can occur. At the beginning of an attack, patients suffer from anxiety and pain, without signs of obstruction or peritonitis on physical examination. The lack of physical signs may result in diagnoses of neurasthenia or hysteria, which can be emotionally traumatic.

The majority of individuals with pathogenic hydroxymethylbilane synthase (HMBS) variants remain asymptomatic, as only a minority (1-10%) of all HMBS gene mutation carriers experience an acute attack during their lifetime.[19] Generally, symptomatic patients do not present before puberty and most patients are females of the reproductive age; however, acute porphyric attacks can occur in both men and women.[5,10] Although most symptomatic patients have only one or two attacks during their lives, a small subset of the symptomatic patients suffer from recurring attacks (≥ 4 hospitalisations due to attacks per year).[20,21] Both symptomatic patients and asymptomatic gene mutation carriers of any of the acute porphyrias have an increased lifelong risk of hypertension, kidney disease, and hepatocellular carcinoma (HCC), but the incidence differs between porphyria types and countries.[22-23]

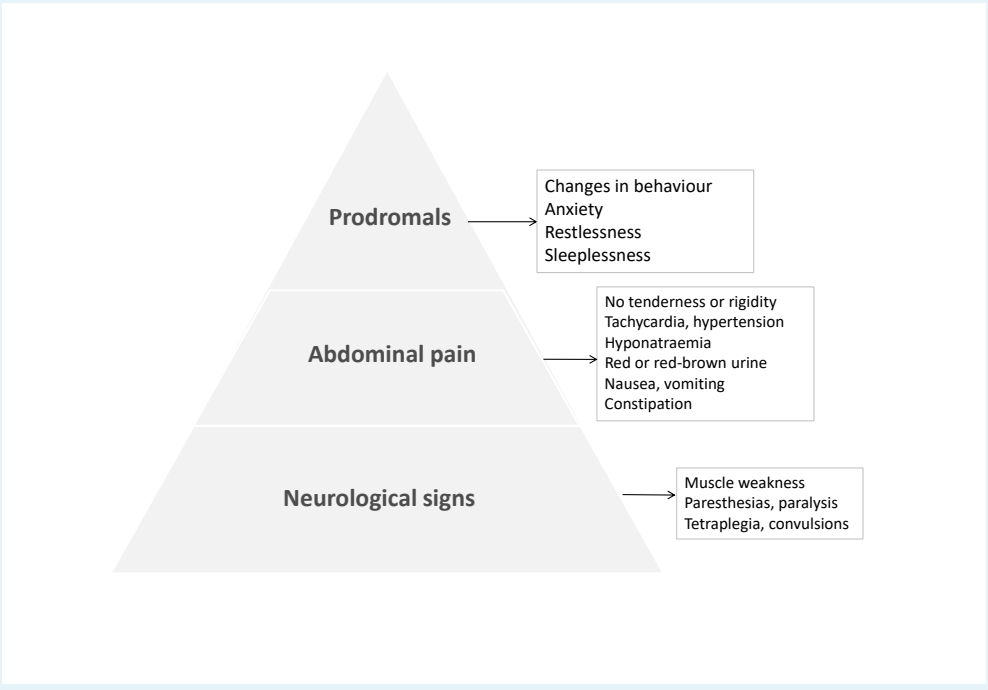
When to suspect an acute porphyric attack

An acute porphyric attack should be considered in all individuals with episodes of severe, unexplained abdominal pain. Since abdominal pain is a very common presenting symptom, identifying other specific symptoms can help direct the differential diagnosis towards an acute porphyric attack. These symptoms are presented in figures 2 and 3. During an attack, some or all features may be present, but there is no single pathognomonic sign for an acute porphyric attack.[4]

What should be investigated?

When an acute porphyric attack is considered, urinary ALA, preferably together with PBG, should be measured. This can

Figure 2. *Characteristic symptoms during an acute porphyria attack.*
Attacks of acute porphyria usually occur in three phases. First, a patient can experience prodromal symptoms; second, abdominal pain with symptoms related to autonomic dysfunction; and finally, a worsening with neurological deficits including muscle weakness, paresthesia, and with ongoing paralysis, seizures, coma, and even death. In some cases, the order can be different. When fever or an increased C-reactive protein level or even a palpable abdominal mass is present, this can be related to the provoking factor but is not caused by the porphyria attack.



be performed in a urine sample; 24-hour collections are not necessary.[24] An acute porphyric attack can be considered when ALA levels (or, if an ALA assay is unavailable, PBG levels) are increased to at least twice the upper limit of normal (ULN). Diagnostic guidelines are in progress in Europe and the United States (US), and there is debate on what the cut-off value should be; twice ULN can be considered conservative and safe regarding false-negative interpretations. Importantly, the urine sample should be protected from light (using aluminium foil) and kept refrigerated (< 4 °C) while being sent to the laboratory. Protecting a sample from light during transport is necessary to prevent false-negative findings, since light stimulates non-enzymatic isomerisation and changing of ALA/PBG into porphyrins and other metabolites. After attacks, urinary ALA and PBG levels can return to baseline levels, but in most AIP patients this normalisation takes years.[25] Plasma ALA and PBG levels are of pivotal importance in patients that are anuric. Plasma ALA/PBG levels can remain elevated much longer than urinary levels after an attack subsides and as such, might be an important biological marker in patients that present asymptomatically but have had suspected attacks in the past.[26-27]

Mild ALA increases (twice ULN) can also be explained by certain drugs, heavy metals such as lead, renal disease, and liver disease.[28] After confirming an acute porphyric attack, treatment should be started without delay. The type of porphyria does not affect the initial treatment, however, following initiation of treatment, further diagnostic steps should determine the specific type of acute porphyria (AIP, VP, HCP, ALAD). For rare cases with acquired porphyria such as lead intoxication, additional steps such as preventing the patient's exposure to the toxicant are essential to cure the patient. There are porphyria expert centres in most European countries, where the required diagnostic tests and the interpretation can be performed.[29] The ALA/PBG ratio, HMBS enzyme activity in erythrocytes, and plasma (or faeces) porphyrin spectrum can guide clinicians to the correct porphyria type. DNA gene mutation analysis can be used as a confirmation step. If a DNA gene mutation cannot be detected, additional enzyme activity can be measured. The specific DNA gene mutation can be used for family counselling. If no mutation in the coding region can be found, a diagnosis can be confirmed by measuring the appropriate enzyme activity (e.g., erythrocyte/lymphocyte porphobilinogen deaminase activity in AIP).

Treatment of an acute porphyric attack [30,31]

Treatment should be started as soon as possible in known acute porphyria patients presenting with severe symptoms, like neurological symptoms, or hyponatraemia, even before the results of confirmatory tests are performed. Treatment should also be started in severe cases without waiting for confirmatory ALA/PBG tests (table 2). In milder cases, without neurological symptoms, it is

Table 2. *Diagnostic test overview and inheritance pattern for all porphyrias*

Name	Cutaneous symptoms on light-exposed skin	Acute attacks	Hepatic or erythroid origin	First diagnostic test*		Inheritance
				(Spot) urine test	Blood test	
AIP	No	Yes	Hepatic	ALA/PBG		Autosomal dominant
VP+	Blistering	Yes	Hepatic	ALA/PBG	Porphyrins in plasma	Autosomal dominant
HCP+	Blistering	Yes	Hepatic	ALA/PBG	Porphyrins in plasma	Autosomal dominant
ADP	No	Yes	Erythro-hepatic	ALA/PBG		Autosomal **recessive**
PCT	Blisters, milia	No	Hepatic	Porphyrins		Autosomal dominant†
EPP	Pain	No	Erythroid		Protoporphyrin IX in erythrocytes**	Autosomal **recessive**††
CEP	Blisters	No	Erythroid	Porphyrins		Autosomal **recessive**
HEP	Blisters	No	Erythro-hepatic	Porphyrins		Autosomal **recessive**

* Urine and blood samples should be protected from light by wrapping in aluminium foil and kept refrigerated until assay.
** Whole blood or erythrocytes are required for measurement.
† PCT is most often caused by multifactorial factors.
†† There is also a rare X-linked form: ALAS2 with gain of function mutations[56,57]
+ VP and HCP can present as acute attacks of abdominal pain, or acute attacks accompanied with cutaneous symptoms, or as solely cutaneous symptoms.
ALA = delta-aminolaevulinic acid; AIP = acute intermittent porphyria; ADP = delta-aminolaevulinic acid dehydratase deficiency porphyria; CEP = congenital erythropoietic porphyria; EPP = erythropoietic protoporphyria; HCP = hereditary coproporphyria; HEP = hepato-erythropoietic porphyria; PBG = porphobilinogen; PCT = porphyria cutanea tarda; VP = variegate porphyria

recommended to start with oral (preferable), and otherwise parental, carbohydrate loading; for example, 200 g or the equivalent, such as 2 litres 10% glucose per day (except in patients with hyponatraemia; they should only receive carbohydrate via the enteral route and haem should be considered immediately). It is also very important to remove any provoking factor(s). Clinical evidence on the effectiveness of a high doses of carbohydrates in suppressing attacks is limited, but there is a good theoretical basis for providing extra carbohydrates as they have demonstrated inhibition of haem synthesis in hepatocytes via PGC-1α (peroxisome proliferator-activated receptor-γ coactivator-1α); carbohydrate-restricted diets are notorious for provoking attacks. During an attack, patients are at risk of developing hyponatraemia and plasma sodium should be initially measured every three hours. Hyponatraemia should be evaluated and treated according to hyponatraemia guidelines. The exact pathogenesis of hyponatraemia in acute porphyrias is not clear; in most cases, findings point towards an inappropriate secretion of antidiuretic hormone and therefore, high-dose glucose infusion should be stopped immediately. Pain control during attacks can be difficult to achieve and high-dose opioids are often needed. In severe attacks and those with significant hyponatraemia, haem arginate should be given immediately. Haem arginate must be dissolved in 20% albumin to prevent phlebitis and given at a dose of 3 mg/kg daily for three to four successive days (one ampoule of haem arginate (Normosang®) contains 250 mg which is sufficient for most adults). The bottle and tubes containing haem need to be protected against light (by aluminium foil wrapping or other). The presence of hyponatraemia, neurological signs, and/or severe non-responsive pain are markers of a severe attack and such patients should be admitted to an intensive care unit.

Haem administration leads to a rapid decrease in ALA and PBG over a period of days, and sufficient pain reduction can take additional time. Peripheral neuropathy and paralysis take weeks to months to improve, but haem therapy can prevent progression to paralysis, seizures, coma, and death.[32]

Hypertension can be exacerbated by the pain, anxiety, and stress, but is also considered to be caused by autonomic dysfunction due to the neurotoxicity of ALA. Beta-blockers, such as propranolol, are safe and effective for reducing blood pressure and tachycardia.

All medication, either prescribed or over the counter, should be cross referenced for safety with the Norwegian Porphyria Centre Drug Database for Acute Porphyria (www.drugs-porphyria.org). In this database, alternative drug options are provided per indication. Drugs that are not safe or possibly harmful, including herbal medications (e.g., St John's wort), should also be discontinued.

Follow-up of symptomatic and asymptomatic patients

In general, patients should be counselled about their disease and informed about lowering their risk of attacks by avoiding provoking factors, including drugs, alcohol, and smoking. All new prescriptions should be checked regarding safety.[33] Surgical procedures warrant pre-operative planning and, in circumstances with risk of low carbohydrate intake, measures should be discussed. Each patient should have an emergency plan and a medic alert, with what to do and monitor in these situations.[34] Blood pressure and renal function should be monitored lifelong on a regular basis. Deterioration of kidney function in AIP patients is independent of hypertension, suggesting a secondary mechanism for kidney damage.[35] One proposed mechanism is the direct toxic effect of ALA, causing tubular atrophy, interstitial fibrosis, and chronic arteriolopathy.[36]

An increased risk for hepatocellular carcinoma (HCC) in acute porphyria patients is well recognised and is unrelated to liver fibrosis or cirrhosis.[37-39] The pathophysiological mechanism has not been elucidated.[40] Considering this risk, it is advised to screen patients over 50 years of age with a bi-annual liver ultrasound, similar to the HCC screening schedule in hepatitis-induced liver cirrhosis patients.

All first-degree relatives should be counselled by a clinical geneticist in a porphyria expert centre,[29] considering their 50% chance of having the same HMBS gene mutation.

Patients with recurrent attacks

A small subset of patients has frequent attacks (≥ 4 per year) that often require hospitalisation. These patients report significantly more porphyria-related symptoms, also in between attacks. They have more chronic complications and a lower quality of life.[7,8] Current treatment options include prophylaxis by weekly haem infusion.[41]

A promising emerging therapy for patients with recurrent attacks is the suppression of ALAS1 activity by administering small interfering RNA (siRNA), givosiran (Alnylam Pharmaceuticals, Cambridge, USA). A phase III trial (ClinicalTrials.gov – NCT03338816) shows impressive and significant reductions in attack frequencies and urinary ALA (Envision, A phase 3 study of safety and efficacy of givorisan, an investigational RNAi therapeutic, in acute hepatic porphyria patients. Gouya L & Sardh E. International Congress on Porphyins and Porphyrias 2019, Milano, Italy). In November 2019, givosiran was approved by the US Food and Drug Administration and in March 2020, by the European Commission/European Medicines Agency (EMA).[42,43] Further studies on the long-term effects and safety of this drug are ongoing; an international phase III trial demonstrated a significant reduction in the number of attacks and levels of ALA and PBG.[81]

A liver transplantation is currently the only available curative treatment for AIP. It replaces the deficient

hepatic enzyme and prevents future attacks, but liver transplantation is not always possible and remains a high-risk procedure.[44-48] Another emerging therapy still in the pre-clinical phase is messenger RNA for AIP patients (mRNA, Moderna Ltd, Cambridge, USA).[49]

Cutaneous porphyrias

The cutaneous porphyrias are porphyria cutanea tarda (PCT); erythropoietic protoporphyria (EPP); and four rare forms, including hereditary coproporphyria, variegate porphyria, and extremely rare, hepato-erythropoietic porphyria (HEP) and congenital erythropoietic porphyria (CEP) (table 3).[50] The symptoms of cutaneous porphyrias include blistering with scarring, milia, hypo-depigmentation, skin fragility, photomutilation, and very painful phototoxicity.[2,51] Figure 3 provides a diagnostic flowchart for distinguishing the main cutaneous porphyrias.

Pathophysiology of cutaneous porphyrias

Porphyrin molecules are formed or modified during the last six steps of haem biosynthesis. Porphyrin molecules are excited by visible light, especially blue light, and to a much lesser degree, UVA light of near violet wavelengths. The porphyrins accumulate in the skin and/or erythrocytes, and exposure to light leads to phototoxic damage by energy released from the porphyrins. The energy released from porphyrins results in the formation of oxygen radicals and causes local haemolysis, histamine release from mast cells, and endothelial damage resulting in tissue damage. In contrast to UV-light, blue light is not absorbed by glass and therefore patients are not protected by glass or by UV sunblocks or sunscreens, which mainly block UV-B light. Patients can only shield themselves from blue light by avoiding exposure and staying indoors, or covering their skin by wearing protective clothing such as long sleeves, long pants, socks, gloves, and broad-brimmed hats. PCT and EPP will be discussed in more detail.

Porphyria cutanea tarda

In PCT, accumulation of the photo-reactive uroporphyrin III in the epidermis causes painless blisters in sunlight-exposed areas of the skin and an increased fragility of the skin. PCT is caused by decreased activity of the hepatic

Table 3. *Symptomatology, prevalence, and inheritance of the cutaneous porphyrias*

Porphyria Type	Age of onset	Cutaneous Symptoms* Present on sunlight-exposed skin	Inheritance	Enzyme	Gene (locus)	Prevalence (cases per million)
PCT sporadic form	> 45 years	Blistering Scarring Milia	Multifactorial: alcohol, iron,** hepatitis C	-	-	~ 40†
familial form	> 25 years		Autosomal dominant	UROD	(1p34.1)	0.2 - 20
EPP	Childhood	Pain Skin oedema, damage, and crusts	Autosomal recessive	FECH	(18q21.31)	9
			X-linked	ALAS2	(Xp11.21)	< 1
HCP	Any age	Blistering Scarring Pigmentation of scars	Autosomal dominant	CPOX	(3q11.2)	0.2
VP	Any age			PPOX	(1q23.3)	3.2
CEP	From birth	Blistering Scarring Mutilation Joint contractures Hypertrichosis	Autosomal recessive	UROS	(10q26.2)	< 1
HEP	From birth	Blistering Scarring Mutilation Joint contractures Hypertrichosis	Autosomal recessive	UROD	(1p34.1)	< 1

*Only on sun-exposed areas of the body, e.g., face, ear concha, and dorsal side of hands. Visible (blue) light is responsible for photoactivation of porphyrins and symptoms. Blue light is not reflected by ordinary glass and therefore does not provide protection. Some EPP patients have symptoms from artificial light, in addition to sunlight.
**HFE test is required after determination of iron overload, as there is a high prevalence of hereditary haemochromatosis in PCT patients.
† Based on[44] and from https://www.porphyriafoundation.org/for-patients/types-of-porphyria/.
Prevalence varies widely between countries. ALAS2 = delta-aminolaevulinic acid-synthase 2; CEP = congenital erythropoietic protoporphyria; CPOX = coproporphyrin oxidase; EPP = erythropoietic protoporphyria; FECH = ferrochelatase; HCP = hereditary coproporphyria; HEP = hepatoerythropoietic protoporphyria; PCT = porphyria cutanea tarda; PPOX = protoporphyrin oxidase; UROD = uroporphobilinogen decarboxylase; UROS = uropoporphobilinogen-synthase; VP = variegate porphyria; XLEPP = X-linked erythropoietic protoporphyria

enzyme uroporphyrinogen-decarboxylase (UROD). This enzyme catalyses the conversion of uroporphyrinogen to coproporphyrinogen. PCT occurs as hereditary or sporadic forms. In sporadic PCT, external provoking factors include alcohol consumption, hereditary haemochromatosis, hepatitis C, smoking, oestrogens, renal insufficiency, exposure to certain chemicals, or a combination of these. The reported association between human immunodeficiency virus (HIV) and PCT is almost certainly due to co-infection with hepatitis C.[52] In hereditary PCT, patients have a pathogenic gene mutation in the UROD gene which causes decreased UROD activity. Only a small percentage of UROD gene mutation carriers will develop symptoms, and external factors are still required to increase uroporphyrin levels and develop symptoms.[53] Hereditary PCT accounts for approximately 25-50% of all PCT cases, with large differences in prevalence between different countries.[54] Hereditary PCT patients present at a younger age, in the third or fourth decade of life, while patients with the sporadic form usually present in their fifties or later. In both forms, there is an almost invariable association with some degree of iron overload.

When to suspect PCT?

PCT should be suspected in patients with painless blisters and lesions on the back of the hands and/or face and the ears, and in late onset hirsutism. The skin becomes fragile and minor trauma or rubbing can easily peel skin off, causing open lesions, crust formation, and eventually scarring and milia. Blisters and scars in skin not exposed to sunlight are very unlikely to be due to PCT.

What should be investigated?

A porphyrin spectrum in a spot urine sample should be tested. Patients with PCT have a specific pattern of porphyrins, with increased levels of uro-, hepta-, and hexaporphyrin (table 2). The urine sample must be protected from light (packed in aluminium foil) and kept refrigerated (< 4 °C) until analysis.

The following tests should be performed to define the provoking factors: renal function, liver enzymes, iron status, hepatitis C, and HIV serology. When raised serum ferritin levels are associated with increased transferrin saturation, diagnostics for hereditary haemochromatosis (HFE gene sequencing) should be performed. In rare cases, high dose oestrogens, haematological malignancy, or liver adenomas can provoke PCT. As there is an increased incidence of liver tumours in PCT, a liver ultrasound is recommended at least once, and more frequently if other risk factors for hepatocellular carcinoma such as cirrhosis are present.[55]

Treatment and follow-up of PCT

Patients with active PCT should stop drinking alcohol, stop smoking, and avoid direct sunlight completely until symptoms resolve and biochemistry is normalised. Patients should consider stopping oestrogen use.

Treatment of PCT is nearly always successful. The treatment consists of eliminating provoking factors and reducing iron stores by phlebotomies or chelation in all patients. Phlebotomies should be continued until urinary uroporphyrin levels are near normal and ferritin is at least < 50 µg/l, whilst maintaining normal haemoglobin levels; this recommendation is based on the Dutch hemochromatosis guideline.[56]

When phlebotomies are contraindicated or not tolerated, hydroxychloroquine can be considered in a low dose (start with 200 mg once a week or 100 mg twice a week).[50] In hepatic lysosomes, hydroxychloroquine forms soluble complexes with the accumulated porphyrins (uroporphyrin) and subsequently increases urinary porphyrin excretion, resulting in a darkening of the urine.[57,58] In addition to increasing porphyrin excretion, it has been suggested that chloroquine and hydroxychloroquine increase urinary iron excretion.[59] High doses of chloroquine and hydroxychloroquine, such as given in rheumatology, must be avoided in PCT as they result in hepatotoxicity.[60] Whether ophthalmological screening is necessary is doubtful as retinopathy has never been described with the low dose and the treatment duration is often less than one year.[61,62] According to the guidelines of the Dutch Society of Rheumatologists, ophthalmological screening should be considered in PCT patients with other risk factors such as renal impairment, concomitant treatment with tamoxifen, or the presence of maculopathy or retinopathy.[63] Most patients achieve biochemical remission within nine months.[57] Some patients continue to experience skin fragility after biochemical remission.

Erythropoietic protoporphyria (EPP)

Erythropoietic protoporphyria patients are intolerant to light. The photosensitivity usually starts in early childhood and is lifelong. EPP is caused by ferrochelatase deficiency (FECH) or by increased activity of the erythroid specific delta-aminolaevulinic acid synthase 2 (ALAS2). In 1-2% of EPP patients, the disorder is caused by a gain of function variant in ALAS2.[64,65] EPP patients accumulate protoporphyrin IX (PPIX) in their erythrocytes. Signs in the skin of EPP patients, such as erythema and oedema may not be immediately visible to the physician when the patient presents with severe pain. The pain and skin damage in EPP patients, is deeper in the skin compared to damage in the skin of a PCT patient. Light exposure of protoporphyrin IX (PPIX) in erythrocytes circulating in small skin vessels results in an energy release with oxygen radical formation, causing endothelial and skin damage. This process results in extreme pain and can occur after a few minutes up to 30 minutes in sunlight. The pain

can last for days after withdrawal from light, and may be followed by erythema, oedema, and petechiae. Blistering is uncommon. The differences in skin changes between EPP and PCT (and HCP and VP) are thought to be due to the localisation of the porphyrins in the skin and possibly in the cell organelles. In EPP, the initial site of damage seems to be in the endothelium and in PCT in the interstitium.

When to suspect EPP?

EPP should be suspected in children and adults who present with severe pain and/or oedema in reaction to light exposure. The majority of patients experience their first symptoms during early childhood. At a very young age, they can present with excessive crying. Children often learn to avoid light even before the diagnosis is made. Most patients describe a prodromal phase, shortly after exposure to (sun)light. This is described as a burning, tingling, or itching sensation in the skin. When light exposure is prolonged, pain will increase, and eventually oedema, erythema, petechiae, necrosis, and crusts may be seen. The pain does not respond to analgesics, including opioids, and can last up to a week. These painful episodes result in light-avoiding behaviour from an early age, with a diminished quality of life, anxiety, vitamin D deficiency, and a negative impact on social life. The amount of time patients can stay in direct sunlight is highly variable between patients and is influenced by weather conditions. Windy, cold sunny days are the worst. EPP symptoms can occur during light exposure behind glass, as blue light is not filtered by glass.

What should be investigated?

To diagnose EPP, free erythrocyte PPIX levels should be elevated by > 3 times the ULN (table 2). The blood sample must be shielded from direct light (packed in aluminium foil) and kept refrigerated but not frozen (4°C). Both free protoporphyrin and zinc protoporphyrin are measured. In addition to protoporphyrin levels, zinc protoporphyrin levels are markedly raised in ALAS2 X-linked protoporphyria, and the ratio can help differentiate between *ALAS2*- and *FECH*-related EPP. Zinc protoporphyrin levels are also increased in iron deficiency and lead poisoning. Most patients will have a loss of function FECH mutation combined with a common polymorphism, which causes mis-splicing and reduced expression of normal mRNA.[64] A diagnosis can be genetically confirmed with *FECH* or *ALAS2* gene mutation analyses (table 2).

Treatment and follow-up of EPP

Until 2016, EPP patients could only avoid light exposure as there was no effective treatment registered for EPP except bone marrow transplantation, which cures EPP but has great health risks. UV-B therapy, beta-carotene, and cysteine have been tried, but effects were unsatisfactory.[67] In 2014, the EMA approved afamelanotide (a synthetic alpha-melanocyte stimulating hormone, Scenesse®, Clinuvel Pharmaceuticals Limited, Melbourne, Australia) marketing authorisation for adult EPP patients in the European Union and in 2016, treatment became available and reimbursed in the Netherlands. Afamelanotide binds to the melanocortin 1 receptor thereby increasing the production of photoprotective eumelanin in the skin. Patients who are currently treated with afamelanotide (subcutaneous implant with 16 mg, 1-4 times per year) report an increase in symptom-free sunlight exposure time, a decrease of the phototoxic effect, and an improved quality of life with only mild adverse events (nausea, headaches, flu-like symptoms, or flushes).[68-69] Afamelanotide treatment can only be given in specialised porphyria expert centres, and is currently available in Switzerland, Italy, Germany, Austria, and the Netherlands. In the remaining European countries, reimbursement issues are still a problem. In the US, the FDA approved afamelanotide in October 2019.[70]

All patients with EPP should be screened for vitamin D deficiency and nearly all require vitamin D supplementation. Osteoporosis and osteopenia are more prevalent in EPP patients and occur at a younger age compared to the general population.[71] Dual-energy X-ray scans are recommended at least once in adult patients.

Approximately 5-20% of patients with EPP develop liver disease.[72] In plasma, free PPIX binds to albumin and is taken up by the liver and excreted via the bile ducts into the faeces. PPIX damages bile duct cells and the hepatic parenchyma. Patients with EPP have an increased risk of gallstones (pigment stones containing protoporphyrin). An EPP-related acute cholestatic hepatitis is the most serious complication of EPP, occurring in fewer than 5% of the patients.[73-75] During a hepatic crisis, PPIX excretion is decreased, resulting in a steep rise of blood and hepatic PPIX levels, causing more liver damage, with a vicious circle leading to more liver damage, haemolysis, and additionally a further rise in PPIX. The treatment of an EPP-related hepatitis should be in close collaboration with an expert centre. One treatment aim is to rapidly lower PPIX levels to stop additional liver damage and improve hepatic recovery. To rapidly lower PPIX levels, erythrocyte exchange transfusions in combination with additional blood transfusions are the best option. Haemoglobin levels should be maintained at levels that suppress endogenous erythropoiesis (preventing the production of PPIX-rich erythrocytes). Liver transplantation can be considered when this therapy fails. As PPIX is produced mainly by the bone marrow and because hepatopathy may recur, a subsequent bone marrow transplantation should be considered.[75-76] An acute cholestatic hepatitis can be precipitated by other causes of liver disease, including

viral hepatitis, hepatotoxic drugs, and excessive alcohol intake.[77] As a preventive measure, all EPP patients are urged to refrain from alcohol and hepatotoxic medication, and immunisation against hepatitis A and B is advised. Caution should be taken for EPP patients requiring surgery, since prolonged exposure (> 3 hours) to bright surgical lights can induce phototoxic tissue injury, which can be prevented by using yellow filters that block blue light.[78-80]

CONCLUSIONS

Awareness of the porphyrias is of great clinical importance as they are a diverse group of conditions with severe consequences and complications. When these diseases remain undiagnosed, serious and irreversible damage can occur. This can often be prevented, and early recognition and treatment of acute porphyric attacks can save lives.

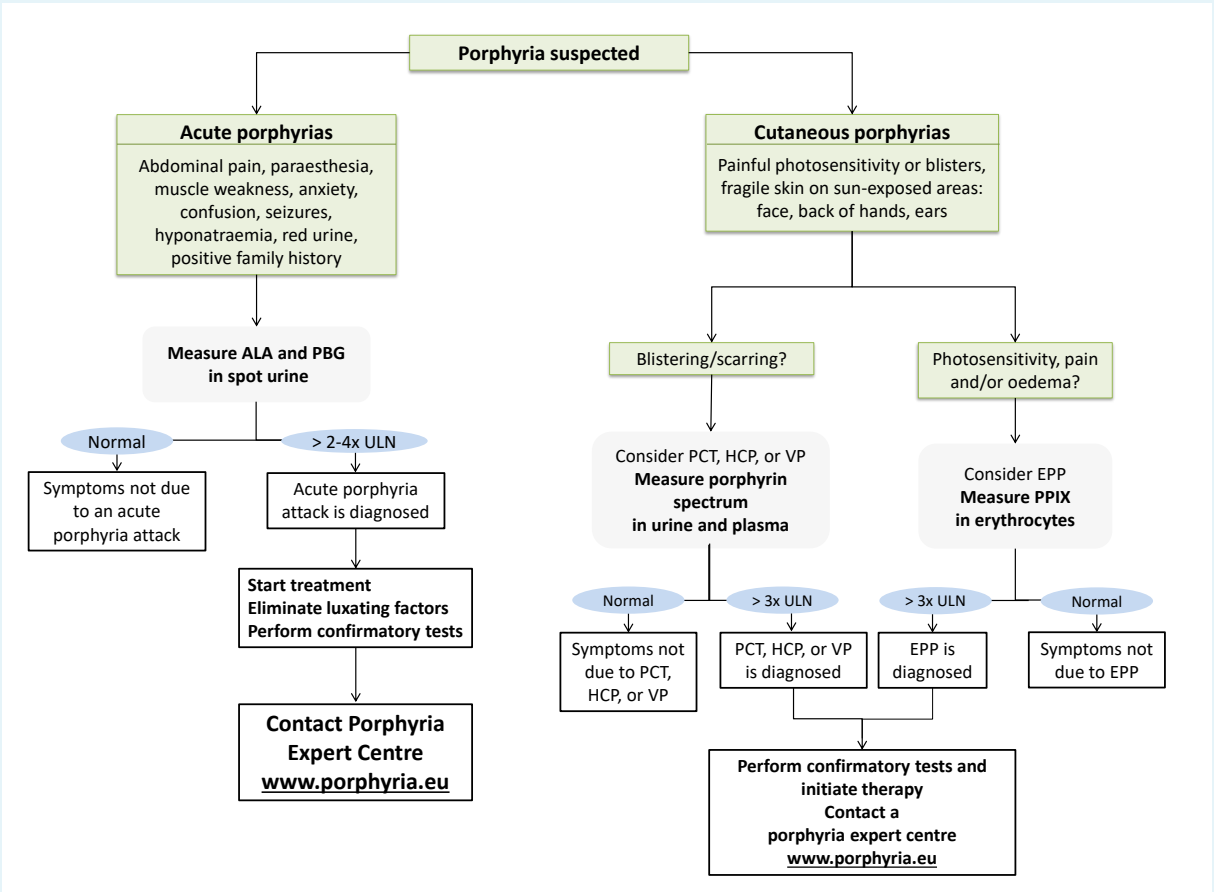
Simple flowcharts are provided to help diagnose porphyria or exclude porphyria from the differential diagnosis (figure 3). Management, treatment, and follow-up of porphyria patients should preferably be performed by or in cooperation with expert centres, and by physicians with experience in treating these disorders (www.porphyria.eu).

Take home messages

Acute porphyria

- Most patients present with severe abdominal pain, combined with one or more of the following 'red flags': paresthaesias, muscle weakness, anxiety, confusion, seizures, hyponatraemia, dark-red urine, and positive family history.
- An acute porphyric attack can be diagnosed by a urinary ALA or PBG measurement > 4 times the upper limit of normal.
- Intravenous haem arginate (Normosang®) is a lifesaving treatment.

Figure 3. *Diagnostic flowcharts for when and how to consider and diagnose acute and cutaneous porphyrias*



ALA = delta-aminolaevulinic acid; EPP = erythropoietic protoporphyria; HCP = hereditary coproporphyria; PBG = porphobilinogen; PCT = porphyria cutanea tarda; PPIX = protoporphyrin IX measured in erythrocytes; ULN = upper limit of normal; VP = variegate porphyria

- All drugs should be checked in the acute porphyria drug database website: www.drugs-porphyria.org before being prescribed to carriers of acute porphyria genes.
- Surgical procedures and other circumstances (infections, etc.) with the risk of low carbohydrate intake should be monitored.
- All acute porphyria patients and carriers should be provided with an emergency plan for acute attacks (www.investof.nl).

Cutaneous porphyrias

- Skin symptoms are related to energy release from blue light-exposed porphyrins, presenting with blisters and skin fragility or pain.
- In patients presenting with severe pain, erythema, and oedema after light exposure, consider erythropoietic protoporphyria (EPP).
- Glass or UV sunblocks do not prevent porphyria-related skin symptoms.
- Afamelanotide (Scenesse®) is a life changing therapy available for adult EPP patients. It improves their light tolerance, reduces and shortens the pain episodes, and increases quality of life.
- Tables 2 provides diagnostic and therapeutic strategies for all porphyrias and genetic counselling advice.

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We recommend the EPNET websites with disease information for physicians and patients (www.porphyria.eu), and the Drug Database for Acute Porphyria (www.drugs-porphyria.org). The INVEST website (www.investof.nl) provides an emergency protocol for an acute porphyric attack.
There are two patient organizations in the Netherlands for patients with porphyrias: www.epp.info (for patients with EPP) and www.pvap.nl (for patients with acute porphyrias).
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DISCLOSURES

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ORIGINAL ARTICLE

Salicylate use: a negative predictive factor for finding pathology explanatory for iron deficiency anaemia

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ABSTRACT

Purpose: To determine whether the use of salicylates is a predictive factor for detecting explanatory pathology during gastroscopy or colonoscopy procedures in patients with iron deficiency anaemia (IDA).

Methods: This retrospective study included patients who underwent a gastroscopy and/or a colonoscopy to determine the cause of IDA at Treant Healthcare, hospital location Scheper in Emmen, the Netherlands, between 2010 and 2016. The study compared two groups. The first group consisted of patients who were not taking antithrombotics at the time of, and during the last six months prior to, the endoscopy. The second group consisted of patients who used salicylates at the time of, and during the last six months prior, to the endoscopy. Data were collected on whether and which explanatory pathology was found in the endoscopic evaluation.

Results: In total, 464 patients were included, of whom, 174 were using a salicylate and 290 were not. In 41.2% of the patients, explanatory pathology was found, which was not significantly different between the two groups with univariate analysis ($p = 0.207$). However, the patients in the group of salicylate users were significantly older and more often male. When correcting for these differences in group characteristics during multivariate analysis, the use of salicylates was found to be a negative predictive factor for finding explanatory pathology ($p < 0.001$; OR 2.307).

Conclusion: When determining the chance of finding explanatory pathology during endoscopic evaluation in patients with IDA, the use of salicylates should be taken into account as a negative predictive factor for finding explanatory pathology during endoscopic evaluation.

KEYWORDS

Colonoscopy, endoscopy, gastroscopy, iron deficiency anaemia, salicylate

INTRODUCTION

When there is no overt bleeding source, iron deficiency anaemia (IDA) is most frequently due to occult gastrointestinal blood loss. An evaluation of the gastrointestinal tract by gastroscopy and/or colonoscopy is therefore the standard in diagnosing the cause of IDA.[1-3] A gastrointestinal lesion explanatory for IDA is found in about two-thirds of the patients who are evaluated with gastroscopy or colonoscopy.[1,4-8] Known positive predictive factors for finding explanatory pathology in endoscopy are higher age, male gender, a low mean corpuscular volume, and a positive faecal occult blood test (FOBT).[9-11]

Salicylates are widely used in low doses to prevent cardiovascular and cerebrovascular disease.[12] Because it is known that salicylates can cause erosions and spontaneous micro-bleeding of the gastrointestinal mucosa,[13-15] it can be hypothesised that salicylates could cause iron deficiency anaemia, even in the absence of visible abnormalities upon gastrointestinal endoscopy. This would make the use of salicylates a negative predictive factor associated with finding explanatory pathology for IDA during an endoscopy. On the other hand, we could assume that salicylates cause pathology like polyps, ulcers, and carcinomas to bleed more quickly, and that the use of salicylates would reveal this pathology earlier, making it a positive predictive factor for explanatory pathology findings. Literature about whether the use of salicylates is a positive or negative predictive factor for explanatory pathology findings for an IDA during gastroscopy or

colonoscopy procedures is scarce and contradictory. Previous studies have concluded that salicylates can cause a small increase of occult gastrointestinal blood loss, although this was clinically irrelevant.[16,17] Some studies found no relation between the use of salicylates and explanatory pathology findings.[8,9] However, in a group of elderly patients with IDA, it was observed that significantly less explanatory pathology was identified in patients who were using salicylates.[18] The review of Banerjee et al. states that in patients who use anticoagulants in general, colon polyps and carcinomas can be revealed earlier compared to patients who are not using anticoagulants.[19]

Knowledge of the impact of salicylate use in determining a clinically-relevant disease in patients with IDA is important as it might influence the pre-test probability of detecting explanatory pathology with a gastrointestinal endoscopy. This is especially important because the procedure is invasive and often performed in elderly and fragile patients. Knowing the pre-test probability of finding explanatory pathology can help physicians with decision making regarding whether to perform a gastrointestinal endoscopy in these groups of patients.

The aim of this study was to determine whether the use of salicylates is a predictive factor for finding explanatory pathology in gastroscopy or colonoscopy in patients with IDA.

MATERIALS AND METHODS

Study population

The study population consisted of patients who underwent a gastroscopy and/or colonoscopy to determine the cause of IDA at Treant Healthcare, hospital location Scheper in Emmen, the Netherlands. A record search was done in the hospital information system to find all patients who were diagnosed with an IDA in the period between January 1st, 2011 and December 31st, 2016. Because an IDA diagnosis was probably changed to a new diagnosis in the hospital information system if a cause was found, a search was also performed for the diagnoses that could be explanatory for IDA. Of this last group, only the patients who underwent a gastroscopy and/or colonoscopy because of an IDA were included. An IDA was defined as a haemoglobin concentration below 8.5 mmol/l for men and below 7.5 mmol/l for women, in combination with a ferritin level below 30 µg/l.[20] In patients with a clear acute process, the ferritin level could not be used because ferritin is also an acute phase reactant.[21] In these cases, a diagnosis was also considered an IDA if, in addition to a low haemoglobin concentration, the mean corpuscular volume was below 80 fl, the transferrin level was above 45 µmol/l, or the serum iron was below 14 µmol/l.

The study compared two groups. The first consisted of patients who were not using antithrombotics at the time of, and six months prior to, the gastroscopy or colonoscopy. The second group consisted of patients who used a salicylate at the time of, and at least during the last six months prior to, the gastroscopy or colonoscopy. Patients were not excluded if their salicylate was temporarily stopped for seven days to reduce the bleeding risk during endoscopy. Patients who used antithrombotics other than a salicylate were excluded. Because of the changed a priori chance, patients were excluded if, prior to the endoscopy, a radiological evaluation or a FOBT was performed. Patients who underwent only one of the endoscopies, either a gastroscopy or a colonoscopy, were included if the reason for not continuing the other endoscopy was the finding of relevant pathology during the first endoscopy. If visibility during endoscopy was poor, due to unsuccessful bowel cleansing or for other reasons, patients were excluded, unless a second successful endoscopy was performed. Most patients underwent biopsies to diagnose coeliac disease. Exceptions were made if the a priori chance of coeliac disease was very low, for example in elderly patients. The decision whether to perform biopsies was individualized and was made by the attending physician. The outcomes of other diagnostic tests performed after the endoscopic evaluation were not included in this study. Because of the increased chance of anaemia, patients with haematological diseases were excluded. Patients with gastrointestinal tract malignancy in their medical history were excluded because of the increased a priori chance for a recurring malignancy and because of the possibility of earlier proceeding to perform an endoscopy in this group of patients, for example to reassure them.

All data of included patients were stored anonymously in a database: gender, age at the time of the endoscopy, the use of salicylates, whether an explanation for the anaemia was found during gastroscopy or colonoscopy, and whether a complication of the gastroscopy or colonoscopy had occurred. The following diagnoses were considered explanatory for an IDA: colorectal malignancy, colon polyps ≥ 1 cm,[19,22] inflammatory bowel disease, angiodysplastic lesions, reflux oesophagitis with erosions that were at least 5 mm long (stage B, C, or D), erosive gastritis, coeliac disease, oesophageal cancer, gastric cancer, small bowel cancer, and peptic ulcers. Like in other studies, diverticula of the colon were not considered explanatory for an IDA.[1,3,4,5,8]

The primary research parameter was in how many cases an explanatory diagnosis was found for IDA during gastroscopy or colonoscopy, and if there was a difference between the two groups. Furthermore, the number of complications was compared between the two groups.

Statistical analyses

A power analysis was conducted with α set at 5% and β at 20%. Based on literature, it was expected that in about two-thirds of the cases an explanatory diagnosis would be found. A difference of 25% between the two groups was considered clinically relevant. The number of patients required was 137 per group.

The statistical analyses were conducted with the program IBM SPSS Statistics version 22. Since age was not normally distributed, a Mann-Whitney test was performed to study the difference in age between the two groups. Dichotomous data (salicylate use, explanatory finding, complications) were compared with the Fisher's exact test. To analyse whether an association existed between independent variables (salicylate use, age, gender) and the outcome variable (explanatory finding), univariate logistic regression analyses were used. To correct for confounders, a multivariate logistic regression analysis was performed. A p-value ≤ 0.05 was considered statistically significant. A receiver operating characteristic curve (ROC curve) was created to determine the diagnostic value of the model used in multivariate logistic regression analysis, and the area under the curve (AUC) was determined. The diagnostic value was considered significant if the 95% confidence interval of the AUC was above 0.5.[23]

RESULTS

In total, 464 patients were included, 174 who used a salicylate and 290 who were not using a salicylate. Table 1 shows the patient characteristics of the total sample

Table 1. *Patient characteristics*

	Total group (n = 464)	Salicylate use (n = 174)	No salicylate use (n = 290)	p-values
Age (med/min-max)	68.5 (17-92)	75 (48-92)	63 (17-86)	< 0.001
Gender **Male, n (%)** **Female, n (%)**	 163 (35.1%) 301 (64.9%)	 76 (43.7%) 98 (56.3%)	 87 (30.0%) 203 (70.0%)	0.004
Explanatory finding*, n (%)	191 (41.2%)	65 (37.4%)	126 (43.4%)	0.207
Complications†, n (%)	2 (0.4%)	1 (0.6%)	1 (0.3%)	1.000

*Number of patients where explanatory pathology for the iron deficiency anaemia was found;
† number of patients with a complication related to the endoscopic evaluation
n = number

Table 2. *Observed pathology in endoscopic evaluation*

Pathology	Total group (n = 464) n (%)	Salicylate use (n = 174) n (%)	No salicylate use (n = 290) n (%)
No pathology	273 (58.8%)	109 (62.6%)	164 (56.6%)
Colorectal malignancy	92 (19.8%)	27 (15.5%)	65 (22.4%)
Colon polyps ≥ 1 cm	10 (2.2%)	4 (2.3%)	6 (2.1%)
Inflammatory bowel disease	1 (0.2%)	0 (0%)	1 (0.3%)
Angiodysplastic lesion	26 (5.6%)	11 (6.3%)	15 (5.2%)
Reflux oesophagitis stage B/C/D	18 (3.9%)	6 (3.4%)	12 (4.1%)
Erosive gastritis	18 (3.9%)	8 (4.6%)	10 (3.4%)
Coeliac disease	9 (1.9%)	1 (0.6%)	8 (2.8%)
Oesophageal cancer	0 (0%)	0 (0%)	0 (0%)
Gastric cancer	1 (0.2%	0 (0%)	1 (0.3%)
Small bowel cancer	1 (0.2%)	0 (0%)	1 (0.3%)
Peptic ulcer	15 (3.2%)	8 (4.6%)	7 (2.4%)

Table 3. *Univariate logistic regression analysis*

	B*	p-value	OR†	[95% CI]‡
Age	0.028	< 0.001	1.029	1.013-1.044
Gender	0.853	< 0.001	2.346	1.589-3.465
Salicylate use	0.253	0.197	1.288	0.877-1.893

*Regression coefficient; †Odds ratio; ‡95% Confidence interval

Table 4. *Multivariate logistic regression analysis*

Variables	B*	p-value	OR†	[95% CI]‡
Age	0.039	< 0.001	1.039	1.021-1.058
Gender	0.858	< 0.001	2.358	1.571-3.539
Salicylate	0.836	< 0.001	2.307	1.467-3.627

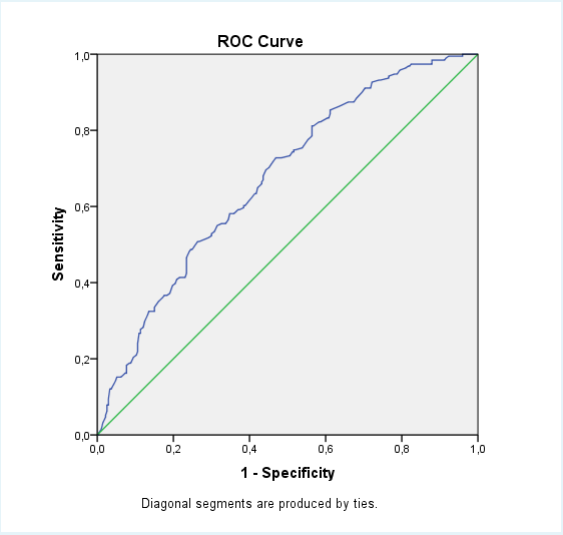
*Regression coefficient; †Odds ratio; ‡95% Confidence interval

and of the separate groups. Median age was significantly higher in the group of salicylate users than in the group of patients who were not using a salicylate ($p < 0.001$). Also, gender was significantly different between the two groups ($p = 0.004$), with significantly more male patients in the group of salicylate users. Explanatory pathology for IDA was found in 41.2% of the patients, which was not significantly different between the group of salicylate users and the group that did not use salicylate. Table 2 shows the identified pathology and frequency per group. A complication occurred in only two of the 464 patients: a perforation after colonoscopy in a patient who used a salicylate and a bleeding after colonoscopy in a patient who did not use a salicylate.

Univariate logistic regression analysis showed that both age and gender were significantly related to finding explanatory pathology at gastroscopy or colonoscopy (table 3). The higher the age of the patient, the higher the chance of finding explanatory pathology, with an odds ratio of 1.029 per year difference in age ($p < 0.001$). Explanatory pathology was significantly more often found in males than in females, with an odds ratio of 2.346 ($p < 0.001$). In univariate analysis, the use of a salicylate was not a significant predictor for finding explanatory pathology.

Since higher age and male gender were predictors for finding explanatory pathology, and since age was significantly higher in the group of salicylate users, which consisted of significantly more male patients, the variables age and gender were identified as possible confounders. A multivariate logistic regression analysis was therefore conducted (table 4), which showed that all variables were significantly related to finding explanatory pathology at gastroscopy or colonoscopy (all $p < 0.001$). After correction for age and gender, the use of a salicylate is, therefore, a negative predictor for finding explanatory pathology for IDA. Explanatory pathology is significantly more often found in patients who are not using a salicylate than in patients who are using a salicylate, with an odds ratio of 2.307. The area under the curve in the ROC curve was 0.673 (95% CI 0.624-0.722) (figure 1), indicating that the model has a significant diagnostic value.

Figure 1. *Receiver operating characteristic (ROC) curve of the multivariate logistic regression model*



DISCUSSION

Occult gastrointestinal blood loss is the most common cause of iron deficiency anaemia when there is no overt bleeding source.[1-3] It is known that salicylates can cause erosions of the intestinal mucosa and an increase in gastrointestinal blood loss.[13-15] This study showed that significantly less explanatory pathology for IDA is found during gastroscopy or colonoscopy in patients with IDA who are using salicylates.

At first, it appeared that no differences existed in the frequency of finding explanatory pathology between the group of patients who were using and the group of patients who were not using a salicylate. However, the salicylate users were significantly older and more often male. Higher age and male gender are known positive predictive factors for finding explanatory pathology in endoscopic evaluation in IDA.[9-11] If salicylate use had no influence on finding explanatory pathology, it was expected that more explanatory pathology would have been found in the group of salicylate users. When correcting for age and gender, multivariate logistic regression analysis showed that the use of salicylates is a negative predictive factor for finding explanatory pathology. In previous studies, no relation between salicylate use and finding explanatory pathology was found.[8,9] The reason for this might be, that in these studies, no corrections were made for age or gender differences in salicylate users and non-users. The outcome of our study not only supports previous findings in elderly patients with IDA, where a relation between salicylate use and a negative endoscopy was found, but also shows that the relationship between the use of salicylates and negative endoscopies is independent of age.[18] Although previous studies stated that an increase in occult gastrointestinal blood loss was not significantly influenced by the use of salicylates, this study suggests the opposite because of more negative endoscopies in salicylate users.[16,17]

A limitation of this study is that the question whether salicylates can cause IDA cannot be answered, because the small intestine, where other explanatory pathology could have been located, was not evaluated with capsule endoscopy. Because the study was retrospective, it is also possible that no corrections were made for variables that could have influenced the probability of finding explanatory pathology, like the exact haemoglobin value or the mean corpuscular volume. In addition, the use of other medication was not taken into account.

Other studies showed that in about two-thirds of the patients, an explanatory finding for IDA is found during gastroscopy or colonoscopy.[1,4-8] In this study, explanatory pathology was found in only 41.2% of the patients. A possible explanation is that, unlike those studies, patients were excluded from our study if a FOBT or radiological evaluation was performed prior to the endoscopic evaluation. It is likely that we may have thus excluded patients in whom explanatory pathology was more likely to be found, since a positive FOBT is a positive predictive factor for finding explanatory pathology, and radiological evaluation also influences the a priori chance for finding explanatory pathology. Another explanation could be that patients with a malignancy of the gastrointestinal tract in their medical history were excluded. It is likely that more explanatory pathology would have been found in this group of patients, due to the increased a priori chance of malignancy.

In conclusion, the use of salicylates should be considered as a negative predictive factor when predicting the outcome of an endoscopic evaluation in patients with IDA. Explanatory pathology for IDA is found significantly less often in patients who are using a salicylate than in patients who are not using a salicylate. Endoscopic evaluation will still be the standard for detecting relevant pathology, also in patients using salicylates. When there is doubt about performing an endoscopic evaluation, for example, in elderly and fragile patients, the use of salicylates should be included in the decision making of whether or not to perform an endoscopic evaluation in patients with IDA.

DISCLOSURES

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

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ORIGINAL ARTICLE

Attitudes of Dutch intensive care unit clinicians towards oxygen therapy

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ABSTRACT

Background: Over the last decade, there has been an increasing awareness for the potential harm of the administration of too much oxygen. We aimed to describe self-reported attitudes towards oxygen therapy by clinicians from a large representative sample of intensive care units (ICUs) in the Netherlands.

Methods: In April 2019, 36 ICUs in the Netherlands were approached and asked to send out a questionnaire (59 questions) to their nursing and medical staff (ICU clinicians) eliciting self-reported behaviour and attitudes towards oxygen therapy in general and in specific ICU case scenarios.

Results: In total, 1361 ICU clinicians (71% nurses, 24% physicians) from 28 ICUs returned the questionnaire. Of responding ICU clinicians, 64% considered oxygen-induced lung injury to be a major concern. The majority of respondents considered a partial pressure of oxygen (PaO_2) of 6-10 kPa (45-75 mmHg) and an arterial saturation (SaO_2) of 85-90% as acceptable for 15 minutes, and a PaO_2 7-10 kPa (53-75 mmHg) and SaO_2 90-95% as acceptable for 24-48 hours in an acute respiratory distress syndrome (ARDS) patient. In most case scenarios, respondents reported not to change the fraction of inspired oxygen (FiO_2) if SaO_2 was 90-95% or PaO_2 was 12 kPa (90 mmHg).

Conclusion: A representative sample of ICU clinicians from the Netherlands were concerned about oxygen-induced lung injury, and reported that they preferred PaO_2 and SaO_2 targets in the lower physiological range and would adjust ventilation settings accordingly.

KEYWORDS

Conservative oxygenation, intensive care unit, mechanical ventilation, oxygen therapy, questionnaire, survey

INTRODUCTION

Optimal oxygen therapy in critical care remains a subject of debate. Almost all critically ill patients receive supplemental oxygen to prevent tissue hypoxia, but the appropriate oxygen dose remains unclear.[1] Recent guidelines recommend targeting an oxygen saturation of 94-98% and stopping oxygen therapy when saturation reaches 96%.[2,3] This recommendation is mainly based on observational studies suggesting harmful effects of liberal oxygen therapy.[4-8] The first randomised controlled trial performed in one Italian ICU found an absolute mortality reduction of 8.6% for critically ill patients when limiting oxygen supplementation titrated to conservative oxygen saturation targets.[9] However, a recent large multicentre randomised controlled trial found no difference in outcomes of adults undergoing conservative or usual oxygen therapy in the ICU, even though targeted oxygenation was frequently not achieved.[10]

In recent years, self-reported views of nurses and physicians with regards to oxygen therapy in critically ill patients have evolved towards a more restrictive approach. In the Netherlands, oxygen-induced lung injury was seen as a major concern.[11] However, in actual clinical practice, these concerns were not accommodated, and the majority of partial pressure of oxygen (PaO_2) values recorded were higher than self-reported targets. Hereafter, conservative oxygenation targets were introduced into daily clinical practice.[5] A recent study showed an increase in the number of ICU clinicians concerned about oxygen-induced lung injury, which was also reflected in actual clinical practice.[12] However, these Dutch studies were performed in three ICUs actively involved in research focused on oxygen therapy for critically ill patients. It therefore remains unknown whether these beliefs are widely supported and whether the results of previous studies can be generalised to ICU clinicians across the Netherlands. In the present study, we aimed to describe self-reported attitudes towards oxygen therapy from a large sample of ICU clinicians across the Netherlands. We further aimed to assess if there were differences in attitudes between nurses and physicians, age categories, and type of ICU.

MATERIALS AND METHODS

Questionnaire

The questionnaire was a translated and comprehensive version of previously used surveys from Canada and Australia/New Zealand, and was also previously used in the Netherlands.[11-14] The anonymous online questionnaire consisted of 59 multiple-choice questions that were designed to elicit self-reported behaviour of ICU clinicians with respect to oxygen therapy in general and in specific case scenarios (complete questionnaire in Dutch: Supplement 1)*. It included questions about oxygen-induced lung injury, risks of mechanical ventilation, indices of tissue oxygenation, and arterial saturation (SaO_2) and PaO_2 targets for short and long-time periods in an acute respiratory distress syndrome (ARDS) patient receiving mechanical ventilation. The last part of the questionnaire investigated whether the respondent would adjust a fraction of inspired oxygen (FiO_2) of 50% for given SaO_2 and for given PaO_2 in the following case scenarios: ARDS, cardiac ischaemia, cerebral ischaemia, sepsis, percutaneous coronary intervention (PCI) stent, and untreatable anaemia.

Target population

In April 2019, 36 ICUs in the Netherlands were approached with the request to send out a web-based questionnaire (LimeSurvey) to their nursing and medical staff. Subsequently, ICU clinicians were invited by email to complete the questionnaire. A reminder was sent out if deemed necessary. The invited ICUs included mixed medical and surgical adult ICUs in university and non-university hospitals across the Netherlands. The ethical reviewing board was informed and had no objection (G18.110).

Statistical analysis

Questionnaire responses are presented as a proportion of respondents per question. Differences in questionnaire responses between nurses and physicians, university and non-university ICUs and age categories were analysed using the Chi square test of Fisher's exact test as appropriate. A p-value below 0.05 was considered statistically significant. Statistical analyses were conducted using R i386 3.4.4.

RESULTS

Characteristics of questionnaire respondents

Between April and August 2019, 1361 questionnaire responses were received from ICU clinicians (61% completed all questions). ICU clinicians from 28 (78%) out of 36 invited ICUs in the Netherlands participated in the online questionnaire. These 28 participating ICUs comprised the majority of all available ICU beds in the Netherlands (428 of 831 beds, data from the 2018 Dutch National Intensive Care Evaluation, https://stichting-nice.nl/datainbeeld/public). Two hundred-twenty-six (19%) ICU clinicians that participated in the questionnaire were from four university hospitals. Respondents consisted of 847 (71%) nurses, 287 (24%) physicians (8% residents, 1% fellows and 15% intensivists), and 53 (5%) with another type of practice (e.g., ventilation practitioner, physician's

Table 1. *Responses to questions regarding concerns, risks, and indices of oxygen therapy and oxygenation for critically ill patients.*

Answers	Total n (%)	Nurses n (%)	Physicians n (%)	University n (%)	Non-university n (%)	> 40 years of age n (%)	< 40 years of age n (%)
Question: Is oxygen-induced lung injury a concern when placing a patient on mechanical ventilation?							
YES, a major concern							
due to the high incidence of injury	46 (4)	31 (4)	12 (4)	8 (4)	38 (4)	21 (3)	25 (5)
due to the severity of injury	434 (38)	335 (42)	79 (28)	77 (36)	357 (39)	232 (36)	202 (41)
due to the high incidence and severity of injury	248 (22)	181 (23)	55 (20)	56 (26)	192 (21)	135 (21)	113 (23)
YES, but not a major concern	358 (32)	224 (28)	121 (43)	69 (32)	289 (32)	226 (35)	131 (27)
NO, it is not a concern	49 (4)	34 (4)	14 (5)	7 (3)	42 (5)	29 (5)	20 (4)
Total number of respondents	1135	805	281	217	918	643	491
		p < 0.01		p = 0.51		p = 0.03	
Question: In your opinion, which one of the following two situations poses a greater threat of lung injury for mechanically ventilated patients?							
High FiO_2	194 (17)	169 (20)	19 (7)	24 (11)	170 (18)	109 (17)	85 (17)
High tidal volumes and high ventilator pressures	953 (82)	649 (78)	261 (92)	198 (88)	755 (80)	531 (82)	420 (82)
Don't know	18 (2)	14 (2)	4 (1)	2 (1)	16 (2)	11 (2)	7 (1)
Total number of respondents	1165	832	284	224	941	651	512
		p < 0.01		p = 0.01		p = 0.90	
Question: In situations when maximum SaO_2 achievable is low (± 85%) or when FiO2 requirements are high, do you assess indices of tissue oxygenation?							
NO	294 (28)	212 (30)	65 (23)	237 (28)	57 (28)	175 (29)	119 (27)
YES, lactate	629 (60)	424 (59)	185 (65)	505 (60)	124 (62)	350 (59)	279 (63)
YES, microcirculation with OPS/SDF imaging	12 (1)	9 (1)	1 (0)	11 (1)	1 (1)	7 (1)	5 (1)
YES, other	108 (10)	72 (10)	32 (11)	89 (11)	19 (10)	66 (11)	41 (9)
Total number of respondents	1043	717	283	201	842	598	444
		p = 0.09		p = 0.85		p = 0.54	
Question: Independent of FiO_2, after what duration would a stable SaO_2 of 85% begin to raise concerns?							
< 2 hours	754 (75)	569 (81)	156 (61)	136 (66)	618 (78)	357 (76)	397 (75)
2-24 hours	184 (18)	108 (15)	66 (26)	42 (21)	142 (18)	82 (17)	102 (19)
24-48 hours	41 (4)	18 (3)	20 (8)	19 (9)	22 (3)	23 (5)	18 (3)
48-72 hours	11 (1)	7 (1)	4 (2)	6 (3)	5 (1)	2 (0)	9 (2)
> 72 hours	10 (1)	2 (0)	8 (3)	2 (1)	8 (1)	8 (2)	2 (0)
Total number of respondents	1000	704	254	205	795	472	528
		p < 0.01		p < 0.01		p = 0.04	

P-values represents difference in questionnaire responses in subgroups, tested with chi square or fisher exact as appropriate. Total number of respondents may differ per question because not all questions were answered by all participants. Number of respondents are shown, with percentages (n (%)).
FiO_2 = fraction of inspired oxygen; OPS = orthogonal polarization spectral.; SaO_2 = arterial oxygen saturation; SDF = sidestream dark field; SvO_2 = mixed venous oxygen saturation.

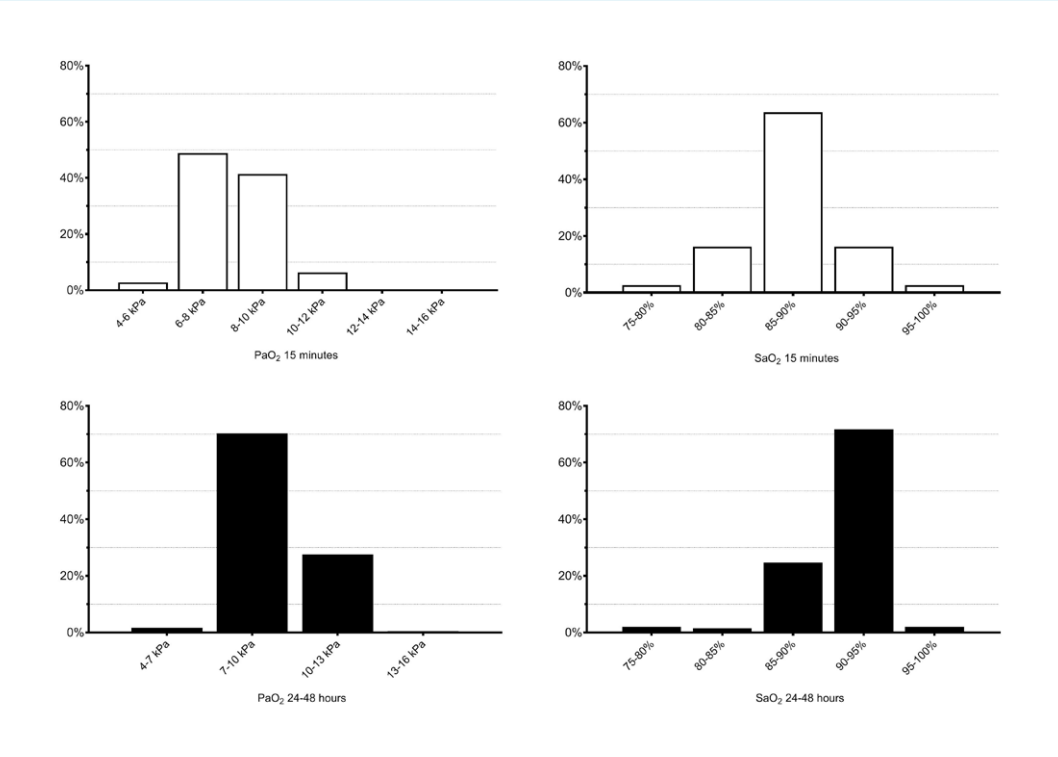
assistant). Of the respondents, 258 (22%) were 18-30 years of age, 374 (32%) 31-40 years of age, 239 (20%) 41-50 years of age, 259 (22%) was 51-60 years of age, and 55 (5%) 61-70 years of age.

Questionnaire responses

The responses to questions regarding concerns when placing a patient on mechanical ventilation are listed in table 1. A majority (64%) of respondents considered oxygen-induced lung injury a major concern when initiating mechanical ventilation; 17% of respondents reported that high FiO_2 posed a greater threat of lung injury than high tidal volumes and high ventilator pressures. Significantly more ICU nurses than physicians considered oxygen-induced lung injury a major concern during mechanical ventilation; 20% of the nurses reported that high FiO_2 posed a greater threat of lung injury than high tidal volumes and high ventilator pressures, compared with 7% of physicians ($p < 0.01$). Compared to nurses, significantly fewer physicians would be concerned with a stable SaO_2 of 85% within two hours (61% physicians vs. 81% nurses, $p < 0.01$). More clinicians from university ICUs responded that high tidal volumes and high ventilator pressures posed a greater threat of lung injury compared to clinicians from non-university ICUs ($p = 0.01$). Compared to university ICU clinicians, more clinicians from non-university ICUs reported they would begin to raise concern after a shorter duration of time with a stable SaO_2 of 85% ($p < 0.01$). ICU clinicians of younger than 40 years of age more often reported they were majorly concerned with oxygen induced lung injury compared to older clinicians ($p = 0.03$).

Figure 1 shows the percentage of respondents accepting various oxygenation ranges in a young to middle-aged mechanically ventilated patient with ARDS. More

Figure 1. *Self-reported acceptable SaO_2 and PaO_2 ranges for 15 minutes and 24-48 hours. Bars represent percentage of respondents. In the questionnaire, respondents were presented with a case of a young-to-middle-aged ARDS patient requiring mechanical ventilation. Ventilator settings are optimised with respect to PaO_2/FiO_2 ratio and haemodynamic indices. There is no evidence to indicate end organ ischaemia, and haemodynamics are stable.*



FiO_2 = fraction of inspired oxygen; PaO_2 = partial pressure of oxygen; SaO_2 = arterial oxygen saturation

Table 2. *Questionnaire responses to questions regarding FiO_2 response for case scenarios with given SaO_2 and PaO_2.*

		FiO_2 response			FiO_2 response		
		Higher n (%)	No change n (%)	Lower n (%)	Higher n (%)	No change n (%)	Lower n (%)
		ARDS			Sepsis		
SaO_2	80-85%	**919 (95)**	51 (5)	1 (0)	872 (98)	12 (1)	3 (0)
	85-90%	**536 (56)**	417 (43)	9 (1)	764 (86)	118 (13)	2 (0)
	90-95%	**9 (1)**	760 (79)	189 (20)	103 (12)	740 (84)	40 (5)
	95-100%	**2 (0)**	107 (11)	865 (89)	6 (1)	257 (29)	629 (71)
PaO_2	6 kPa	**791 (97)**	26 (3)	0	785 (99)	7 (1)	1 (0)
	9 kPa	**175 (22)**	610 (75)	31 (4)	316 (40)	462 (58)	13 (2)
	12 kPa	**6 (1)**	281 (35)	528 (65)	16 (2)	434 (55)	340 (43)
	16 kPa	**2 (0)**	18 (2)	801 (98)	1 (0)	45 (6)	752 (94)
		Cardiac ischaemia			PCI stent		
SaO_2	80-85%	**934 (99)**	7 (1)	1 (0)	869 (99)	8 (1)	0
	85-90%	**874 (94)**	53 (6)	0	813 (93)	61 (7)	0
	90-95%	**310 (33)**	601 (64)	23 (3)	139 (16)	707 (81)	26 (3)
	95-100%	**15 (2)**	528 (56)	396 (42)	4 (1)	299 (34)	578 (66)
PaO_2	6 kPa	**793 (99)**	7 (1)	0	785 (99)	9 (1)	1 (0)
	9 kPa	**488 (61)**	307 (38)	7 (1)	417 (53)	368 (46)	9 (1)
	12 kPa	**31 (4)**	512 (64)	255 (32)	19 (2)	471 (60)	301 (38)
	16 kPa	**3 (0)**	95 (12)	706 (88)	2 (0.3)	51 (6)	746 (93)
		Cerebral ischaemia			Untreatable anaemia		
SaO_2	80-85%	**903 (99)**	12 (1)	2 (0.2)	809 (93)	62 (7)	1 (0)
	85-90%	**829 (92)**	72 (8)	4 (0.4)	705 (81)	154 (18)	7 (1)
	90-95%	**129 (14)**	743 (82)	30 (3)	413 (48)	378 (44)	63 (7)
	95-100%	**6 (1)**	314 (34)	595 (65)	56 (6)	509 (58)	308 (35)
PaO_2	6 kPa	**775 (98)**	12 (2)	2 (0.3)	759 (96)	32 (4)	1 (0.1)
	9 kPa	**345 (44)**	431 (55)	12 (2)	524 (66)	260 (33)	12 (2)
	12 kPa	**16 (0)**	452 (57)	320 (41)	125 (16)	485 (61)	185 (23)
	16 kPa	**3 (0)**	52 (6)	740 (93)	16 (2)	197 (25)	584 (73)

All clinical situations represent patients in the ICU, who have been invasively mechanically ventilated for at least 5 days, with FiO_2 set at 50%.
ARDS: patient with acute respiratory distress syndrome and pneumonia; cardiac ischaemia: patient with signs of cardiac ischaemia (ST-depressions in het anterior leads (max 3 mm) and pneumonia; cerebral ischaemia: patient with recent cerebral ischaemia and one-side hemiplegia; sepsis: patient with liver abscess and sepsis; untreatable anaemia: Jehovah's Witness with stable haemoglobin of 1.8 mmol/l after gastric bleeding; Higher: increase FiO_2 higher than current 50%; no change maintain FiO_2 at current 50%; Lower: decrease FiO_2, lower than current 50%. Number of respondents are shown, with percentages (n (%)).
FiO_2 = fraction of inspired oxygen; PaO_2 = partial pressure of oxygen; SaO_2 = arterial oxygen saturation

physicians reported that lower SaO_2 and PaO_2 were acceptable, compared to nurses (supplementary table 1*, $p < 0.01$). More university ICU clinicians chose lower SaO_2 ranges as lowest acceptable for under 15 minutes and 24-48 hours, compared to non-university clinicians ($p < 0.01$). Older ICU clinicians reported lower PaO_2 as acceptable for up to 15 minutes ($p = 0.01$), compared to younger clinicians.

The proportions of ICU clinicians adjusting FiO_2 levels in different case scenarios are listed in table 2. For the case

scenarios, most respondents reported they would increase FiO_2 if SaO_2 was 80-85% or if PaO_2 was 6 kPa (45 mmHg). If PaO_2 was 16 kPa (120 mmHg), most respondents (≥ 73% of respondents per case scenario) reported they would lower FiO_2. The majority of ICU clinicians reported in most case scenarios, that they would leave FiO_2 unchanged if SaO_2 was 90-95% or if PaO_2 was 9 or 12 kPa (68 or 90 mmHg). Respondents favoured lower SaO_2 and PaO_2 levels for the ARDS cases. Overall, nurses, more frequently than physicians, would increase FiO_2 if SaO_2 or PaO_2 was lower. Physicians more often responded that they would decrease FiO_2 or leave it unchanged if SaO_2 or PaO_2 was lower, except for the untreatable anaemia case scenarios (supplementary table 2). Minor differences were found between university and non-university clinicians and between ICU clinicians < 40 years and > 40 years of age (supplementary tables 3 and 4).

DISCUSSION

This national questionnaire study assessed clinicians working in the majority of ICUs across the Netherlands and showed that ICU clinicians consider oxygen-induced lung injury a major concern; high ventilator pressures and high tidal volumes were considered a greater threat than high FiO_2. For shorter periods of time, ICU clinicians accepted SaO_2 levels as low as 85% and PaO_2 levels as low as 6 kPa (45 mmHg), but a higher limit of 90% and 7 kPa (53 mmHg) is preferred if the situation lasts longer. It seems that ICU clinicians consider a PaO_2 of 6 kPa (45 mmHg) as too low, a PaO_2 of 16 kPa (120 mmHg) as too high, and a PaO_2 of 12 kPa (90 mmHg) as optimal, because they reported adjusting ventilation settings accordingly. In general, compared to nurses, physicians had a more open attitude towards conservative oxygen therapy and would allow lower SaO_2 and PaO_2 levels without adjusting FiO_2. Older and university ICU clinicians would accept lower oxygenation, possibly due to more experience or more awareness of the potential adverse effects.

To our knowledge, this is the first nation-wide study describing self-reported attitudes towards oxygen therapy of ICU clinicians working in the 28 ICUs consisting of the majority of available beds and admissions in the Netherlands. Previous studies have focused on a selection of ICUs nationally or internationally.[11,13,15,16] Perhaps, these ICUs were more oxygen-focused and this could have influenced the questionnaire results. We believe our results are a better reflection of the general attitudes of ICU clinicians towards oxygen therapy in daily critical care. The first study assessing attitudes of intensivists was performed in Canada in 1999 and found that 51% of respondents considered oxygen-induced lung injury a major concern.[14] In our cohort of ICU clinicians, 64% reported oxygen-induced lung injury to be important, which was similar to results of a survey in 2013 of 90 ICU nurses and physicians in Australia.[15] However, in 2010, 542 critical care nurses from Australia and New Zealand were surveyed and only 22% considered oxygen-induced lung injury a major concern.[16] In our cohort, nearly 70% of ICU nurses considered it a significant concern. Apparently, the number of clinicians considering oxygen-induced lung injury in daily practice has increased over the years. This is most likely due to increasing evidence about oxygen-induced lung injury and the effect of conservative oxygen therapy on patient outcomes. However, apart from a time-dependent effect, it may also reflect a geographical difference or difference by chance. In a recent Dutch questionnaire study, performed after the implementation of a conservative oxygenation protocol in three ICUs, 76% of respondents considered oxygen-induced lung injury to be a major concern[12], which was an increase of nearly 20% compared to the assessment before the implementation,[11] and 10% more than what we found in this study. This supports our hypothesis that in ICUs where oxygen-related research is conducted clinicians are more concerned about oxygen-induced lung injury than in other ICUs.

Seventeen percent of the current respondents considered high FiO_2 to be a greater threat of lung injury than high tidal volumes and ventilator pressures, which was similar to 13% previously found.[13,14] This is remarkable, as evidence for lung-injury by high tidal volumes is well accepted,[17] while evidence for the risks of high FiO_2 in ICU patients is still controversial.[9,10] Compared to an earlier study,[16] we found that fewer nurses considered barotrauma to be a greater threat than high FiO_2, compared to physicians. Possibly, physicians may be more often convinced by evidence of ventilator-induced lung injury due to high tidal volumes and pressures.[18-20]

The preferred PaO_2 ranges for short and long time periods in an ARDS patient reported by the current respondents were comparable to the PaO_2 range previously recommended in studies in ARDS patients by the ARDS Clinical Trials Network.[21] These findings were similar to an earlier survey performed in critical care physicians from seven northern European countries where the majority chose a PaO_2 of 10 kPa (75 mmHg) for an ARDS patient.[22] Our questionnaire results also suggest that physicians tolerate lower PaO_2 and SaO_2 values than nurses. Physicians may be more comfortable with lower oxygenation and with taking actions and accountability with the risk of hypoxia. Physicians may also be better informed about the potential downsides of supplemental oxygen therapy. In a previous survey of nurses, more experienced nurses were more likely to answer that they would never be concerned with a stable SpO_2 of 90%.[16]

Our study has the following clinical implications. As this study reflects the beliefs and attitudes of a representative

sample of ICU clinicians from the Netherlands, the results may be useful to customise training, clinical decision making, and protocols. Furthermore, our study provides data for determining PaO_2 and SaO_2 targets in future interventional or observational studies. The current study also gives insight into the differences in attitudes of clinicians. These differences in attitudes could be explained by variances in education and training and by barriers experienced by the clinicians. When implementing new oxygenation strategies in daily critical care, it is important to acknowledge these differences of attitudes and actively engage and educate all clinicians to improve team compliance.

The primary strength of our study is its size and thereby its representativeness. The participation rate of 78% of the invited ICUs was high. In addition, participating ICUs consisted of more than half of the available ICU beds and more than half of all ICU admissions in the Netherlands in 2018. Moreover, the distribution of nurses and physicians was representative of a typical staff constitution of ICUs in the Netherlands. The questionnaire strongly resembles questionnaires previously used in other studies, allowing for comparisons and exploring trends over time and continents.[11,13,14] Furthermore, our results show what clinicians think about oxygen therapy in critical care and for specific pathologies, which could be helpful when reviewing the impact of guidelines for critical care or specific pathologies.

Our study has some limitations. The questionnaire data is self-reported and may not reflect actual practice and does not reflect the practice of non-responders. It has been shown that self-reported attitudes towards oxygen therapy are generally more conservative than actual practice.[11] The cases included in the survey do not represent the complexity of patients in daily practice. SaO_2 and PaO_2 ranges and values in the survey were chosen arbitrarily. Because this was an explorative study, we chose not to correct for multiple testing.

In conclusion, our study provides new insights into the attitudes of ICU clinicians towards oxygen therapy across the Netherlands. The majority of ICU clinicians reported concern about oxygen-induced lung injury and preferred PaO_2 and SaO_2 targets in the lower physiological range. Physicians reported being more conservative with oxygen therapy and decreased FiO_2 at lower SaO_2 and PaO_2 values, compared to nurses.

DISCLOSURES

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

**The supplementary information (Dutch questionnaire / S-tables 1-4) is available upon request; please contact the corresponding author.*

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ORIGINAL ARTICLE

Blood pressure variability within a single visit and all-cause mortality

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ABSTRACT

Background: Within-visit variability of repeated sequential readings of blood pressure (BP) is an important phenomenon that may affect precision of BP measurement and thus decision making concerning BP-related risk and hypertension management. However, limited data exist concerning predictive ability of within-visit BP variability for clinical outcomes. Therefore, we aimed to investigate the association between the variability of three repeated office BP measurements and the risk of all-cause mortality, independent of BP levels.
Methods: Data collected through the National Health and Nutrition Examination Survey (NHANES) were analysed. NHANES is a program of studies designed to assess health and nutritional status of adults and children in the United States. A complete set of three sequential BP measurements, together with survival status, were available for 24969 individuals (age 46.8±19.3 years, 49% males). Multivariable logistic regression models were used to determine the prognostic ability of the examined demographic, clinical, and haemodynamic indices. Results: Among various examined indices of variability of systolic (SBP) and diastolic (DBP) blood pressure measurements, the standard deviation of DBP (DBP_{SD}) was the stronger independent predictor of mortality (odds ratio 1.064, 95% Confidence Interval: 1.011-1.12) after adjustment for age, sex, body mass index, smoking, SBP, heart rate, history of hypertension, diabetes mellitus, hypercholesterolaemia, and cardiovascular events.
Conclusion: Within-visit variability of three sequential office DBP readings may allow for the identification of high-risk patients better than mean SBP and DBP levels. The predictive value of within-visit BP variability and methods to improve its clinical application are worthy of further research.

KEYWORDS

Blood pressure variability, epidemiology, haemodynamics, risk factors, standard deviation

INTRODUCTION

For nearly a century, office blood pressure (BP) measurement has been the basis for hypertension evaluation and management, along with the main principles and techniques of BP recording,[1] which rely on BP measurements, using a brachial cuff-based sphygmomanometer.[2]
However, BP readings may vary considerably, not only in the long term, but also in the very short term (i.e., beat to beat or within a single visit),[3-5] affecting the precision of BP assessment and decision making

about BP-related risk and hypertension management. To address the variation between BP readings, the European guidelines for the management of arterial hypertension recommend that three sequential office BP measurements should be recorded after five minutes of rest in a seated position with the back and arm supported, while additional measurements are required if the first two readings of systolic BP differ by > 10 mmHg, and the average of the last recorded BP measurements should be used.[6] Similarly, other international hypertension recommendations, including the American and Canadian guidelines, recommend the use of the average of two or more BP readings.[7,8]

Although BP variability, within 24 hours, day-to-day, and visit-to-visit, has been extensively evaluated and associated with cardiovascular events and mortality.[9,10] limited and inconsistent evidence exists,[2,11] particularly on the assessment and clinical impact of BP variability during a single medical visit.[12] Therefore, in the present study, we aimed to investigate the predictive ability of within-visit variability for all-cause mortality, over and above BP levels.

MATERIALS AND METHODS

The present study used data from the National Health and Nutrition Examination Survey (NHANES), which is a stratified multistage probability survey conducted in non-institutionalised individuals in the United States, and was administered by the National Center for Health Statistics (NCHS).[13] Details on the NHANES have been described elsewhere.[14] The following data were extracted and used in our analysis: age, sex, smoking status, history of hypertension, diabetes mellitus, hypercholesterolaemia, myocardial infarction, angina, coronary heart disease, congestive heart failure, and stroke. Medical history was self-reported during the medical history interview.[15]

Mortality data

The NCHS has linked various surveys, including NHANES, with death certificate records from the National Death Index (NDI), providing an opportunity to conduct a vast array of outcome studies designed to investigate the association of a wide variety of health factors with mortality. In the present study, we used these data to explore the predictive ability of specific facets of systolic (SBP) or/and diastolic (DBP) short-term variation occurring within a triplicate of sequential measurements of BP levels.

Blood pressure measurement

BP was measured using a specific protocol and equipment described in detail at the 'Physician Examination - Procedures Manual' of NHANES.[16] BP and heart rate measurements were performed using a stethoscope and an inflation system including an arm BP cuff and a sphygmomanometer. The inflation system consists of a latex inflation bag, a Calibrated® V-Lok® cuff, a Latex Inflation Bulb, and an Air-Flo® Control Valve. The pressure gauge is a Baumanometer® calibrated mercury true gravity wall model. All equipment was regularly subjected to quality control checks. A stopwatch was used to standardise the time between consecutive BP readings.

All subjects were asked if they had consumed any food, coffee, alcohol, or if they smoked in the past 30 minutes before the examination. Although intake of coffee, cigarettes, and other vasoactive substances could affect BP, this information was not used to exclude subjects from BP measurements in the NHANES survey.

BP was measured in the right arm. If a BP measurement was not possible in the right arm due to specific known or self-reported conditions which prohibited the use of the right arm, then the measurements were performed in the left arm. All measurements were taken in a sitting position and after at least a five-minute resting period. In order to obtain an accurate BP reading, the appropriate cuff size was determined for each subject following a standardised procedure.[16]

Three sequential BP measurements were obtained on the same arm. If a BP recording was interrupted for any reason, a fourth measurement was obtained. There was a minimum time-interval of at least 30 seconds between repeated BP measurements.

Analysis of within-visit variation of three repeated BP measurements

The degree of SBP and DBP variation of three repeated measurements was quantified using various statistical indices.

Standard deviation of the three SBPs (SBP_{SD}) and DBPs (DBP_{SD}) repeated measurements was calculated using the following equation.

$$x\_BP_{SD} = \sqrt{\frac{\sum_{i=1}^{N}(x\_BP_i - \overline{x\_BP})^2}{N-1}}$$

where N = 3 measurements, x = systolic or diastolic BP, and the average of the three SBP or DBP values.

Variance of the three SBPs (SBP_{VAR}) and DBPs (DBP_{VAR}) repeated measurements was calculated using the following equation.

$$x\_BP_{VAR} = x\_BP_{SD}{}^2 = \frac{\sum_{i=1}^{N}(x\_BP_i - \overline{x\_BP})^2}{N-1}$$

Coefficient of variation of the three SBPs (SBP_{CV}) and DBPs (DBP_{CV}) measurements was calculated as the ratio of x_BP_{SD} to the mean value of the three x_BP measurements where x = systolic or diastolic BP.

Maximum absolute difference (MAD) of SBP (SBP_{MAD}) and DBP (DBP_{MAD}) was expressed as the maximum absolute difference between any two readings among the three repeated sequential SBP and DBP measurements, respectively.

Statistical analysis

The correlations between continuous variables were evaluated by Pearson correlation coefficient. Collinearity between continuous variables was assessed by the variance inflation factor and tolerance determined via multiple regression models. To assess differences among the three repeated sequential BP measurements, we performed general linear models for repeated measurements with Least Significant Difference test for post-hoc analysis of multiple comparisons. Multiple logistic regression models were also used to identify independent factors predicting all-cause mortality. Backward, step-wise modelling was initially used as a first exploratory approach. Finally, various multivariable models were constructed using the enter method. Sensitivity and specificity of indices of BP variation for the prediction of mortality were determined by Receiver Operator Characteristic (ROC) curves.

Finally, we evaluated the incremental prognostic performance of DBP variability on top of the core model of established risk factors for all-cause mortality by calculating: a) the difference in area (s) under the curve (AUC) from corresponding ROC curves, b) the categorical net reclassification index NRI (NRI) as previously described,[17] and c) the integrated discrimination improvement index (IDI). The core model consisted of age, sex, heart rate, BMI, traditional risk factors for cardiovascular diseases (CVD) (e.g., smoking, diabetes, hypertension, hyperlipidaemia), and already adjudicated CVD. All of these factors are well-established factors of increased all-cause and CV mortality as previously reported[18-22] and therefore may act as confounders in the association between DBP within-visit variability and all-cause mortality. Given the lack of pre-defined risk categories of all-cause mortality in our research population, event rate was used to derive the cut-offs implemented in the NRI analysis. Statistical significance was accepted for p-values < 0.05. Statistical analysis was conducted by IBM SPSS Statistics for Windows, (Version 25.0. Armonk, NY: IBM Corp.) and STATA package, version 11.1 (StataCorp, College Station, Texas USA).

Table 1. *Demographic and clinical characteristics of the study population*

Parameter	Total population
N	24969
Age (years)	46.8 ± 19.3
Sex (male)	12247; 49
Weight (kg)	79.9 ± 19.9
Height (cm)	168 ± 10
BMI (kg/m^2)	28.4 ± 6.3
Heart rate (bpm)	72.6 ± 12.4
Smoking status (yes)	2549; 47.1
Hypertension (yes)	7470; 30
Diabetes mellitus (yes)	2443; 9.8
Hypercholesterolaemia (yes)	6658; 42.1
Congestive heart failure (yes)	669; 2.9
Coronary heart disease (yes)	936; 4.1
Angina (yes)	700; 3
Myocardial infarction (yes)	979; 4.2
Stroke (yes)	802; 3.5
All-cause mortality	1937; 7.8

BMI = body mass index; bpm = beats per minute; N = number. Continuous variables are expressed as mean ± standard deviation and categorical variables as absolute frequency (n) and percentage (%).

RESULTS

A complete set of three sequential BP measurements, together with survival status, was available for 24969 individuals (all above 17 years of age). A total of 19012 subjects were free of history of any CVD or cancer. Time to event was not available in the current dataset. Demographic and clinical characteristics of the examined populations are reported in table 1.

Differences among three sequential BP readings

Descriptive characteristics of single, average, and variability indices of SBP and DBP measurements are reported in table 2. Analysis of variance for repeated measures (corrected for multiple comparisons) indicated that the mean value of SBP for the total population varied significantly from measure-to-measure ($p < 0.001$). Specifically, the first SBP value was significantly decreased in the 2nd measurement (SBP_2) by 1.3% ($p < 0.001$) while the difference between the third (SBP_3) and the first (DBP_1) measurement was -2.1% ($p < 0.001$). SBP_3 was also significantly lower than SBP_2 by 0.8% ($p < 0.001$).

Table 2. *Descriptive analysis of systolic (SBP) and diastolic (DBP) blood pressure readings and indices of their variability among the three repeated measurements for the total population*

Parameter	Total population
SBP_1 (mmHg)	124.5 ± 20.0
SBP_2 (mmHg)	122.9 ± 19.2
SBP_3 (mmHg)	121.9 ± 18.8
SBP_{AV} (mmHg)	123.1 ± 19.0
SBP_{SD} (mmHg)	3.7 ± 2.4
SBP_{VAR} (mmHg2)	19.9 ± 28.2
SBP_{CV} (%)	3.0 ± 1.9
SBP_{MAD} (mmHg)	7.1 ± 4.7
DBP_1 (mmHg)	70.5 ± 12.2
DBP_2 (mmHg)	70.0 ± 12.1
DBP_3 (mmHg)	69.7 ± 12.1
DBP_{AV} (mmHg)	70.0 ± 11.7
DBP_{SD} (mmHg)	3.3 ± 2.2
DBP_{VAR} (mmHg2)	15.5 ± 30.2
DBP_{CV} (%)	4.9 ± 3.9
DBP_{MAD} (mmHg)	6.2 ± 4.2

AV = average of three SBP and DBP measurements; CV = coefficient of variation; DBP = diastolic blood pressure; MAD = maximum absolute difference; SBP = systolic blood pressure; SD = standard deviation; Subscripts 1, 2, and 3 correspond to the 1st, 2nd, and 3rd blood pressure recording.; VAR = variation

Similarly, the DBP significantly varied and gradually decreased within the three repeated BP readings ($p < 0.001$), but to a lesser extent than SBP (F_{ANOVA} for SBP = 2430 and F_{ANOVA} for DBP = 294). Specifically, DBP_2 was decreased by 0.7% and DBP_3 by 1.1% compared to DBP_1 ($p < 0.001$). The difference between DBP_3 and DBP_2 was -0.4% ($p < 0.001$).

Mortality and within-visit variation of BP measurements

We initially performed an exploratory multivariable logistic regression analysis, using backward (step-wise) method for the determination of the stronger independent predictors of total mortality (table 3); these were age, sex, heart rate, SBP, DBP_{SD}, history of diabetes mellitus, hypercholesterolaemia congestive heart failure, coronary heart disease, and stroke (table 3). Concerning the within-visit variability of three repeated measurements of DBP, it was observed that an increase of DBP_{SD} by one unit (SD) was associated with 6.4% increased odds of all-cause mortality. Importantly, when DBP_{CV} replaced DBP_{SD}, it remained a significant independent parameter in the model, indicating that the increased variation of DBP can predict mortality regardless of the mean DBP level ($DBP_{CV} = DBP_{SD}/DBP_{average}$). In that case, increase of DBP_{CV} by 1% was associated with 3.6% increased odds of all-cause mortality.

Finally, we constructed several multivariable logistic regression models (enter method) to determine the independent predictors of all-cause mortality after adjustment for various independent variables such as demographic and clinical parameters, and average SBP and DBP. In each model, only one variability index of SBP and DBP (i.e., SD, variance, coefficient of variation, or maximum absolute difference) was entered. It was found that DBP_{SD} was an independent significant predictor of all-cause mortality as reported in table S-1 (Supplementary information)*. Demographic and clinical characteristics of study population by quartile of DBP_{SD} are reported in table S-5 (Supplementary information).

Reclassification

DBP variability conferred incremental reclassification value over the core model for prediction of all-cause mortality (overall NRI = 0.454, $p < 0.001$). Using DBP_{SD}, 210 subjects who died were correctly reclassified into a higher risk category, while 118 participants were falsely stratified as high risk (table S-2, Supplementary information). In addition, increased DBP variability improved the discriminative ability of the core model for the prediction of all-cause mortality (overall IDI = 25.2, standard error = 1.1, $p < 0.001$).

Association of within-visit BP variability with age and other risk factors

We examined which factors, including age, are related with enhanced variability of SBP and/or DBP within a triplicate of sequential BP readings. The association of age on within-office BP variability was assessed by Pearson correlation coefficients (table S-3, Supplementary information). All indices of within-visit variability of SBP were significantly and positively correlated with age, albeit all correlations were weak ($r < 0.3$). The DBP variability indices showed an even weaker association with age ($r < 0.02$).

We further developed eight multivariable linear regression models with each variability index used as dependent variable and age, together with other demographic and clinical parameters as independent variables. After adjustment (enter method) for all other independent factors, age was significantly and positively associated with all SBP variability indices, while heart rate was inversely associated with all SBP variability indices (table

Table 3. *Multivariable logistic regression model (final 'backward' model) of significant independent predictors of all-cause mortality for the total population and for individuals without history of any cardiovascular disease (CVD) or cancer*

TOTAL POPULATION				
Independent variables	***p***	***Odds ratio***	**95% CI - *Lower***	**95% CI - *Upper***
Age (years)	< 0.001	1.085	1.074	1.096
Sex (females)	< 0.001	0.608	0.476	0.778
Congestive heart failure (yes)	< 0.001	3.136	1.976	4.977
Coronary heart disease (yes)	0.020	1.593	1.075	2.361
Stroke (yes)	0.002	2.012	1.285	3.152
Hypercholesterolaemia (yes)	0.016	0.741	0.581	0.947
Diabetes mellitus (yes)	0.002	1.590	1.181	2.139
Heart rate (bpm)	0.001	1.017	1.007	1.027
SBP (mmHg)	0.003	1.009	1.003	1.015
DBP_{SD} (mmHg)*	0.017	1.064	1.011	1.120
WITHOUT CVD/CANCER				
Independent variables	***p***	***Odds ratio***	**95% CI - *Lower***	**95% CI - *Upper***
Age (years)	< 0.001	1.089	1.075	1.102
Sex (females)	0.001	1.744	1.258	2.417
Smoke (yes)	0.035	0.706	0.511	0.976
Hypercholesterolemia (yes)	0.040	0.708	0.509	0.985
Diabetes mellitus (yes)	< 0.001	2.071	1.388	3.092
Heart rate (bpm)	0.003	1.020	1.007	1.034
DBP_{SD} (mmHg)**	0.009	1.090	1.021	1.163

Bmp = beats per minute; CI = confidence intervals; DBP_{SD} = standard deviation of three repeated diastolic blood pressure measurements; p = p-value; SBP = systolic blood pressure.

*Coefficient of variation of three repeated DPB readings (DBP_{CV}) was also an independent predictor of mortality (odds ratio 1.036, p = 0.017) when entered the model instead of DBP_{SD}.

**Maximum difference of DBP (DBP_{MAD}) was an independent predictor of mortality (odds ratio 1.045, p = 0.012) when entered the model instead of DBP_{SD}. DBP_{CV} was a marginally non-significant predictor of mortality (p = 0.057).

S-4, Supplementary information). In contrast, within-visit variability indices of DBP were inversely related with age, and positively correlated with heart rate; overall DBP variability presented lower standardised correlation coefficients (beta) compared to those between SBP within-visit variability and age. Overall, SBP and DBP within-visit variability were associated with BP levels (table S-4, Supplementary information).

DISCUSSION

To the best of our knowledge, this is the first demonstration that within-visit variability of three sequential DBP measurements is an significant predictor of all-cause mortality, independent of demographic and clinical characteristics. Specifically, an increased SD of three repeated DBP measurements (DBP_{SD}) is associated with greater odds of all-cause mortality after adjustment for relevant confounders.

Short-term (24-hour, day-to-night, representing exaggerated circadian BP variations), medium-term (day-to-day) and longer-term (visit-to-visit) BP variability have been associated with increased cardiovascular risk and mortality in subjects with and without hypertension and irrespective of a baseline cardiovascular risk.[23,24] However, limited evidence exists examining the clinical relevance of within-visit BP variability. Li et al. measured BP three times at 5-minute intervals with the use of a validated semi-automated electronic device in 1222

subjects.[25] It was reported that within-visit DBP variability, as measured by the maximum absolute difference (MAD) between any two BP readings, was related to increased carotid intima-media thickness and internal carotid plaque in the normotensive population, whereas SBP_{MAD} was associated with internal carotid plaque in hypertensive patients under antihypertensive therapy, after adjustment for established cardiovascular risk factors.[25]

Another study aimed to evaluate the association between within-visit SBP and DBP variability and the development of pre-diabetes and diabetes longitudinally.[26] Variability of SBP/DBP was assessed using the maximum difference between the three BP measures. It was demonstrated that there was a 77% higher progression to pre-diabetes/diabetes over a three-year follow-up period for individuals with high, compared to low, within-visit SBP variability, independent of major risk factors.[26] In another study, type-2 diabetes mellitus and pre-diabetes were associated with slightly greater within-visit variability (as measured by SD) of both SBP and DBP.[27]

In a large-cross subclinical survey (BP-CARE, Blood Pressure control rate and CArdiovascular Risk profilE) of 6425 treated hypertensive patients living in Eastern European countries,[28] within-visit variability of SBP (but not of DBP) was quantified as coefficient of variation and SD of three repeated SBPs within a single visit. Elevated within-visit SBP variability was associated with several cardiovascular risk including metabolic syndrome, resistant hypertension, impaired renal function, an increased calculated cardiovascular risk, and a higher rate of previous cardiovascular events.[28] Furthermore, increased within-visit variability of SBP and DBP was associated with an increased risk of stroke in a post-hoc analysis of the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA).[29]

Overall, the above-mentioned evidence suggests that within-visit BP variability, assessed by a simple and inexpensive approach, is associated with target organ damage and cardiovascular factors. However, there is limited and controversial evidence concerning the predictive ability of within-visit BP variability for all-cause mortality.[12]

Within-visit BP variability and mortality

Similar to our study, Muntner et al[30] analysed the data from 15317 individuals from the NHANES III survey (3848 deaths), aiming to explore the association between within-visit variability of SBP/DBP and all-cause as well as cardiovascular mortality. Within-visit BP variability (assessed by the SD of three repeated SBP and DBP measurements) was not associated with an increased risk of all-cause or cardiovascular mortality.[30] In our study, we also observed that within-visit variability of SBP could not predict mortality, however, DBPSD (and other indices including DBP_{VAR}, DBP_{CV}, DBP_{MAD}) independently predicted all-cause mortality. Nevertheless, in our study a much larger population with a greater percentage of events (deaths) was examined, using a quite different set of independent variables for the adjustment of the predictive models.

In another study, five consecutive BP readings were obtained from a randomly recruited Flemish population of 2944 subjects.[31] Within-visit SBP variability did not have any prognostic significance over and beyond mean SBP. Nonetheless, in a subgroup analysis, within-visit SBP variability was an independent predictor of the study's endpoints in women and patients on antihypertensive drug treatment.[31] Within-visit DBP variability readings were not analysed.

Hara et al, using a double-blind design, randomly allocated 4695 patients (≥ 60 years) with isolated systolic hypertension to active treatment or matching placebo and investigated whether on-treatment SBP level, visit-to-visit variability, or within-visit BP variability predicted total or cardiovascular mortality.[32] Increased SBP level, but not SBP variability, predicted mortality, while BP-lowering treatment reduced cardiovascular complications by decreasing the level, but not the variability of SBP.[32] However, the analysis of this study was limited to SBP measurements.[32]

Limitations

The findings of the present study should be interpreted within the context of some potential limitations. Although several complex indices and formulas have been described in the literature for the quantification of BP variability, we used the most common and traditional metrics of variability (i.e., SD, variance, coefficient of variation, maximum difference between measurements) in our analyses. In the NHANES survey there was no standard, fixed, time-interval between repeated BP measurements; a minimum interval of at least 30 seconds was applied. Although, specific drug usage was not analysed in our multivariable models, we found that adjusting models for antihypertensive treatment did not alter our findings (data not shown). Finally, the current data cannot provide any causative role or mechanisms through which the reported increase in within-visit DBP variation is linked to elevated risk. As we are moving to unattended office BP measurement using automated or semi-automated electronic BP devices that reduce the white coat effect, the predictive ability of within-visit BP variability needs to be further evaluated.

Nonetheless, it should be highlighted that evidence suggests that short-term BP variations are more pronounced when autonomic reflex cardiovascular control is impaired,[28,33] arterial stiffness is increased,[34] and responsiveness of the central nervous system to environmental and emotional stimuli is enhanced.[28,35]

CONCLUSION

In the era of precision medicine, the findings of this study may have pertinent clinical implications. Within-visit DBP variability assessment through a triplicate of sequential office BP readings may allow the identification of high-risk patients more accurately than SBP and DBP levels alone. Since multiple BP readings are already integrated into everyday clinical practice, the analysis of within-visit BP variability might be an additional simple and cost-effective way to augment precision in risk assessment. In this context, the prognostic value of BP variability within one visit and methods to improve its clinical application are worthy of further research.

DISCLOSURES

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

**The supplementary information (S-tables 1-5) is available upon request; please contact the corresponding author.*

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SPECIAL REPORT

Atypical and fulminant presentations of pneumococcal infections:

A case series in a tertiary intensive care unit.

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ABSTRACT

With the introduction of conjugate pneumococcal vaccines, changes in causative serotypes and clinical presentations of *Streptococcus pneumoniae* infections are occurring. During the 2017-2018 winter, an unusual number of patients with a severe manifestation of pneumococcal disease was admitted to a tertiary care intensive care unit (ICU) in the Netherlands. We describe some of the cases in depth.
Given our observed change in infecting serotypes and extreme clinical manifestations of pneumococcal disease, a systematic clinical registry of pneumococcal infections in the ICU may be a valuable addition to pneumococcal disease surveillance.

KEYWORDS

Atypical presentation, ICU, pneumococcal infection

INTRODUCTION

Streptococcus pneumoniae is a Gram-positive bacterium and a coloniser of the upper respiratory tract. Common clinical manifestations of pneumococcal infections are acute otitis media, pneumonia, and meningitis. However, uncommon manifestations like infections of bone and joint or manifestations affecting the cardiovascular, gastrointestinal, and (uro)genital tract have been described.[1]
Invasive pneumococcal disease (IPD) is defined as positive culture of material from a normally sterile body site (e.g. blood, cerebrospinal fluid, pleura, joint, pericardium).
Pneumococcal disease has a significant burden on mortality and healthcare budget,[2-4] however since the beginning of the 21st century conjugate vaccines have been used to diminish this burden. A decreasing incidence of IPD predominantly in children, ensued the implementation of PCV10 (10-valent pneumococcal conjugate vaccine, directed against serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F) in the paediatric immunisation program.[5] Because children are a reservoir for circulating pneumococci, their immunisation also confers herd immunity in the adult population.[6]
However, existing conjugate vaccines target up to 13 out of more than 95 known pneumococcal serotypes. In most adult populations, the vaccine-mediated reduction in IPD has now been replaced by non-vaccine serotypes.[7-12] Several studies suggest that this shift in causative serotypes also affects clinical manifestations of diseases.[13-16]
It is well known that pneumococcal disease has a seasonal distribution like other respiratory infections, with the highest prevalence in autumn and winter. Temporal associations with seasonal respiratory viruses are described in the literature and pneumococcal superinfections to influenza have been studied in vitro and in animal models.[17-20]
During the respiratory season of 2017-2018, an unusually high number of patients with notable pneumococcal infections was admitted to the intensive care unit (ICU) of our tertiary care centre. In this paper, we present a case series of severe pneumococcal infections and highlight five cases with either uncommon or fulminant manifestations of pneumococcal disease.

METHODS

Case identification and reporting

We searched the hospital digital patient data system and the clinical microbiology data system for adult

patients admitted to mixed medical and surgical ICUs of our academic tertiary care centre with a confirmed or suggested invasive pneumococcal infection between September 1st, 2017 and April 30th, 2018. Patients were considered eligible if *S. pneumoniae* was identified in cultures, or by PCR on materials from normally sterile body sites, or by a pneumococcal antigen test (PAT) (Alere Binex Now, Abbott, USA) on urine at the hospital's clinical microbiology laboratory. In addition, patients referred from other hospitals were identified by text mining of electronic patient records from patients who had been admitted to the ICU during the study period, on synonyms of *S. pneumoniae* infection (Software: CTcue, Amsterdam, the Netherlands). Synonyms included any phrase containing pneumokok, pneumococ, *Streptococcus pneumoniae, S. pneumoniae, or S.pneumoniae*, and all hits were verified manually. Only medical cases with evident pneumococcal aetiology as the main reason for ICU admission were included, excluding patients who were temporarily monitored in the ICU, e.g., for diagnostic procedures (such as bronchoscopy or pericardial drainage). We reviewed patient records and summarised characteristics of individual cases. Serotyping of cultured pneumococcal isolates was performed at the Netherlands Reference Laboratory for Bacterial Meningitis by Quellung reaction. Five cases with atypical or fulminant disease are described in detail.

There is a lack of literature reports on pneumococcal IPD manifestations in the ICU. As a best alternative to appraise our observed distribution of clinical manifestations and mortality, we compared our data to a cohort of all adult pneumococcal bacteraemia cases admitted to the ICU of the neighbouring and a major referring secondary care training hospital (Canisius-Wilhelmina Hospital, Nijmegen, the Netherlands) between 2001 and 2015. For this comparator cohort, details on inclusion criteria, data collection, and representativeness of cohort characteristics (including serotype distribution) for the Dutch population were previously described elsewhere.[21]

CASE SERIES

Case 1: Meningitis

Patient 1 was a 48-year-old man admitted to the ICU with septic shock and meningitis. His medical history listed hypertension and type 2 diabetes mellitus. Two days before admission, symptoms started with an earache. The day before admission, the patient was nauseous and later disoriented. There was no history of fever or meningeal irritation. On the day of admission, the patient was found unresponsive with urinary and faecal incontinence. A Glasgow Coma Scale (GCS) of 6 with uniformous and responsive pupils were reported on presentation and patient was promptly intubated and sedated by the mobile medical team at his home.

Computed tomography (CT) at the emergency room (ER) showed findings suggestive of left-sided mastoiditis with transverse sinus thrombosis. Relevant blood analysis showed signs of an infection with leucocytosis ($31x10^9$/l) and elevated C-reactive protein (192 mg/ml). Blood gas analysis showed a combined metabolic-respiratory acidosis with impaired oxygenation (pH 6.98; partial pressure of carbon monoxide (pCO_2) 7.3 kPa; partial pressure of oxygen (pO_2) 10.2 kPa; bicarbonate (HCO^{3-}) 10.4 mmol/l; lactate > 15 mmol/l). Blood cultures were taken and empirical antimicrobial therapy was started for suspected meningitis with amoxicillin 2000 mg intravenous (IV) three times a day and ceftriaxone 2000 mg IV two times a day (BID), according to hospital guidelines.

After admission to the ICU, a lumbar puncture was performed. Opening pressure was > 50 cmH_2O. Cerebrospinal fluid (CSF) showed 1243 leucocytes/µl (98% neutrophils), protein 3508 mg/l, glucose 2.0 mmol/l (glucose ratio CSF/blood 0.11) and L-lactate 26,260 µmol/l. Gram stain showed Gram-positive cocci in pairs and pneumococcal antigen testing on liquor was positive, after which antibiotic treatment was de-escalated to ceftriaxone monotherapy (because of selective decontamination of the digestive tract, a third generation cephalosporine was given). Nonetheless respiratory status deteriorated and the patient was placed in a prone position. After several hours, both pupils became unresponsive and dilated; a second CT scan of the head showed diffuse swelling of the brain. On the second day of admission, sedation was stopped. GCS remained 3 on day three with the absence of brain stem reflexes. It was decided to withdraw treatment because of a very poor prognosis. Both blood and cerebrospinal fluid cultures yielded growth of *S. pneumoniae*.

Case 2: Peritonitis

Patient 2 was a 38-year-old woman with a medical history of epilepsy, autoimmune pancreatitis, and hepatitis with chronic liver failure. Two days before admission to the ICU, she underwent an endoscopic ultrasound procedure to perform a biopsy of the pancreas. The day after, she complained of severe abdominal pain and was admitted to the hospital. An ultrasound-guided puncture for ascites was performed and cultures were taken. A CT scan showed signs of hepatic ischaemia, an oedematous pancreas and signs of duodenitis, jejunitis, and colitis. Antibiotic treatment was initiated promptly with ceftriaxone 1000 mg IV every day (QD), metronidazole IV 500 mg QD, teicoplanin 12 mg/kg IV BID (to cover *Enterococcus spp*) and anidulafungin IV 100 mg QD (as empirical choice for potential invasive candidiasis). The patient was admitted to the ICU two days after the endoscopic procedure with

sepsis and liver failure. An exploratory laparotomy was performed revealing a diffusely ischaemic jejunum and colon. Because of the extent of the ischaemic lesions, no resection was performed; the abdomen was left open because of high intra-abdominal pressure. The patient developed multiple organ failure (MOF). Culture from the abdominal fluid (ascites) showed *S. pneumoniae* and antibiotic treatment was de-escalated to penicillin 6,000,000 U/day. At ICU day 15, severe rectal blood loss occurred from a rectal ulcer. Because of a lack of treatment options and progression of multiple organ failure, palliative therapy was started. The patient died on day 17.

Case 3: Pneumonia, pleural empyema, pericarditis

Patient 3 was a 68-year-old man with a medical history of a chronic pancreatic insufficiency and a transient ischemic attack (TIA) eight years before admission. The patient was transferred to our ICU after he had been treated for severe pneumonia in another hospital for seven days. He was mechanically ventilated from admission and his stay was complicated by recurrent atrial fibrillation/flutter and cardiogenic shock because of cardiac tamponade. Percutaneous pericardial drainage was performed in the referring hospital without apparent effect. Loculated pleural fluid was present bilaterally and a pleural drain had been placed in the left pleural cavity. Blood cultures were positive for *S. pneumoniae*.

On admission to our hospital, the patient was treated with cefotaxime 1000 mg IV QID. The same evening, a surgical subxiphoidal pericardial drainage was performed. Antibiotic treatment was de-escalated to penicillin 12,000,000 U/day. A sternotomy was performed two days after primary drainage during which, pericardial adhesions were dissected and a partial pericardiectomy was performed, and pleural spaces were opened with debridement of pleural adhesions. Soon thereafter however, the patient developed progressive MOF and it was decided to withdraw active treatment. Patient died on day 11 after primary admission. While pleural and pericardial fluids remained culture-negative, the presence of *S. pneumoniae* in these specimens was later confirmed by PCR.

Case 4: Meningitis, endocarditis

Patient 4 was a 67-year-old woman with a medical history of hypertension, hypercholesterolaemia, type 2 diabetes mellitus, and irritable bowel syndrome (IBS). The patient was admitted to the ICU of the referring hospital with a GCS of 9, a fever of 40 °C, and hypotension. She had reported pain in her left shoulder. The day before admission, she had become acutely ill with pain spreading to her left leg; she also had pollakisuria and mild diarrhoea. A lumbar CSF puncture showed an increased pressure (> 50 cm H2O), high glucose, high protein, high leucocyte count, all compatible with bacterial meningitis. Blood and CSF cultures revealed *S. pneumoniae*. Antibiotic treatment with penicillin 12,000,000 U/day was started. The next day, her neurological status improved. However, there were signs of a recent myocardial infarction on electrocardiography. A screening echocardiogram showed a moderate left ventricular function. Patient had two episodes of acute congestive heart failure and she developed atrial fibrillation. A follow-up echocardiogram showed vegetations on the mitral valve. The patient was intubated and a transoesophageal echocardiogram showed vegetations on both the mitral and aortic valves. Antibiotic treatment with benzylpenicillin 12,000,000 U/day was continued based on the pneumococcal isolate minimum inhibitory concentration of benzylpenicillin of 0.016 mg/l. Because of the poor clinical condition, acute surgical treatment was decided against and conservative treatment with antibiotics was continued. The third day after referral, the patient was extubated and transferred to the ward. A magnetic resonance imaging of the cerebrum showed multiple lesions consistent with infarctions, compatible with septic embolism. Because her condition improved, a mitral valve replacement and coronary artery bypass graft were performed almost one month after first hospitalisation. Culture of the native mitral valve showed no bacterial growth. During hospitalisation, hypogammaglobulinaemia was found, which may have increased the patient's susceptibility for the invasive pneumococcal infection. Gamma globulin treatment was started and continued at home. The antibiotic regime was continued for six weeks.

Case 5: Pneumonia

A 26-year-old woman was transferred to our ICU for venovenous extracorporeal membrane oxygenation (VV-ECMO). She had a medical history of exercise-induced asthma, allergic rhinitis, and migraines. One day earlier, she was admitted to the referring ICU with bilateral pneumonia after she had been ill for a week. The general practitioner had prescribed steroids for asthma exacerbated by a (suspected) viral infection. She was intubated for acute respiratory failure. The following day, she was transferred to our hospital with persistently high respiratory support. Empirical antimicrobial treatment included ciprofloxacin, ceftriaxone, and oseltamivir. After starting prone positioning, respiratory support could gradually be reduced and ECMO support was not required after all. Pneumococcal urinary antigen test was positive, as well as blood cultures showing *S. pneumoniae*. A PCR for influenza A was also positive. Treatment was de-escalated to benzylpenicillin 6,000,000 U/day. During admission, a chest CT scan showed a cavitation in the middle lobe without signs of abscess or of an empyema. *Aspergillus fumigatus* was cultured in a respiratory surveillance specimen; anidulafungin 100 mg QD and voriconazole

160 mg BID were started for suspected influenza-associated invasive aspergillosis. Her respiratory status improved and just over two weeks after admission to our ICU, she was transferred back to the referring ICU. At that point, she was still intubated and on pressure support ventilation, receiving anidulafungin and voriconazole as antimicrobial therapy.

RESULTS

Fifteen cases met our inclusion criteria; 10 from in-house microbiology results and five solely by text mining (table 1). Their age ranged from 26 to 78; eight patients were younger than 65 years old. Eight patients were male and seven patients were female. The mortality in our cohort was 47%.

In three cases, only a pneumococcal urinary antigen test was positive; in one case, only sputum culture. In one case of pericarditis, pneumococcal aetiology was established by PCR on pericardial fluid in combination with a positive pneumococcal antigen test on both urine and a non-determined positive blood culture. In all other cases, *S. pneumoniae* was cultured from blood, cerebrospinal fluid, or ascites. All cultured pneumococci were susceptible for penicillin. In five patients, PCR on influenza was performed; two patients tested positive for type A influenza. Interestingly, the 2017-2018 influenza season was dominated by type B influenza.

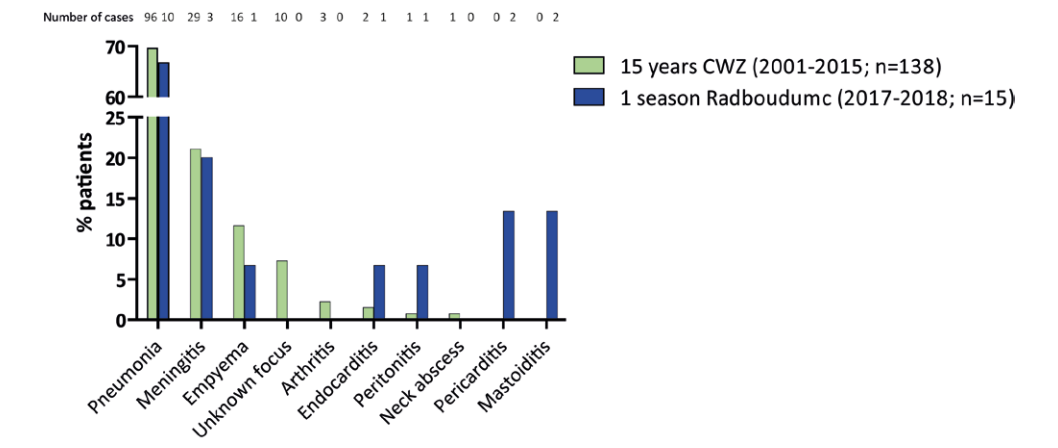
In our population, 11 out of 15 patients had one or more known risk factors such as chronic lung disease (COPD more than asthma), smoking, diabetes mellitus, or chronic heart disease, predisposing them to invasive pneumococcal disease. Two patients had two risk factors. However, three out of seven patients who died had none of these risk factors. Five patients had been treated with some form of immunosuppressant medication. In one patient, severe hypogammaglobulinaemia was identified, making the patient more susceptible to pneumococcal infections. Vaccination status could not be retrieved in any of the patient records. In our cohort, seven out of eight serotyped isolates were non-PCV10 serotypes (table 1).

In our study period of 2017-2018, we observed relatively many uncommon clinical manifestations in our own ICU, in comparison to 138 adult bacteraemic IPD cases admitted to the ICU of a neighbouring secondary care hospital during 15 preceding years (figure 1). None of the seven external patients referred to our academic ICU during the current study period came from the comparator hospital. Mortality was 47% (7 out of 15) in our cohort, compared to 28% (38 out of 135) in the preceding cohort. In the preceding cohort, deaths were significantly older than survivors (mean of 69 versus 62 years old, respectively), and were mainly attributable to pneumonia, meningitis, and patients with an unknown focus of infection (23, 6, and 9 cases, respectively). Although in the current study cohort both deaths and survivors were relatively young (62 and 60 years old, respectively), mortality was particularly high among pneumonia cases (4 out of 9, compared to 23 out of 96 in the preceding cohort).

DISCUSSION

Our case study describes an unusual number of atypical manifestations and/or fulminant character of

Figure 1. *Distribution of clinical manifestations among patients with pneumococcal infections admitted to the intensive care unit in a preceding (green) and current (blue) study cohorts.*



CWZ = Canisius-Wilhelmina Hospital

Table 1. *Cases of pneumococcal disease during respiratory season 2017-2018*

Clinical presentation	Gender	Age (years)	SOFA score (at 24h)	Apache score (at 24h)	ICU admission (days)	Death	Course in the ICU	Medical history	Immuun-suppresive medication	Positive diagnostic test	Serotype	Influenza	Duration of complaints before admisson
Myringitis, mastoiditis, meningitis (Case 1)	M	48	11	28	3	yes	Invasive mechanical ventilation, vasopressors	Type 2 diabetes mellitus, hypertension	No	Blood culture, urine antigen test, cerebrospinal fluid antigen test	8	Not tested	2 days
Peritonitis (Case 2)	F	38	5	28	19	yes	Invasive mechanical ventilation, vasopressors, CRRT, MOF	Epilepsy, autoimmune pancreatitis, acute on chronic liver failure	Tacrolimus, prednisone	Ascites culture	Unknown	Not tested	1 day
Pneumonia	F	57	5	27	10	no	Invasive mechanical ventilation, vasopressors	Asthma, surgery for renal cell carcinoma	Prednisone (not recent)	Blood culture, sputum culture	8	Negative	Hours
Pneumonia	M	78	3	31	24	yes	Invasive mechanical ventilation, vasopressers	Metastatic prostate carcinome	Palliative chemotherapy	Blood culture, urine antigen test	8	Not tested	1 day
Pneumonia	M	76	3	14	7	yes	Invasive mechanical ventilation, vasopressors, inotropics, CRRT	Ulcerative collitis, deep venous thrombosis, upper GI surgery, cardiomyopathy, pacemaker implantation, cholecystectomy	Prednisone (low dose)	Urine antigen test	Unknown	Not tested	Several days
Pneumonia	M	64	6	30	10	no	Invasive mechanical ventilation, vasopressors, chemotherapy	Type 2 diabetes mellitus, COPD Gold 3, hypertension, anal carcinoma (resection and chemoradiotherapy), open abdominal aneurysm repair, pressure ulcer	No	Sputum culture	Unknown	Not tested	Several days
Pneumonia (Case 5)	F	26	11	26	16	no	Noninvasive and invasive mechanical venti, prone positioning, vasopressors	Allergic rhinitis, asthma, migraine	No	Blood culture, urine antigen test	3	Influenza A positive	1 week
Pneumonia, pericarditis, empyema (Case 3)	M	68	11	26	11	yes	Invasive mechanical ventilation, vasopressors, CRRT	Transient ischemic attack, hypertension, total hip replacement, pancreatic insufficiency	No	Blood culture, urine antigen test, PCR pericardial and pleural fluid	8	Negative	Unknown

Clinical presentation	Gender	Age (years)	SOFA score (at 24h)	Apache score (at 24h)	ICU admis-sion (days)	Death	Course in the ICU	Medical history	Immuun-suppresive medication	Positive diagnostic test	Serotype	Influenza	Duration of complaints before admisson
Pericarditis	M	67	9	17	42	no	Invasive mechanical ventilation, vasopressors, CRRT	Alpha thalas-semia, insuline dependent type 2 diabetes mellitus, hyper-trofic cardiomy-opathy, pericar-dial fluid	No	PCR peri-cardial fluid, Gram stain and antigen test-posi-tive blood culture, urine antigen test	Unknown	Negative	Unknown
Pneumonia	F	55	8	19	13	no	Invasive mechanical ventilation, vasopressors	COPD Gold 3, heroin abuse, chronic hepatitis A and B, deep venous thrombosis, urosepsis, hepa-tomegaly, hemi-nefrectomy	No	Urine antigen test	Unknown	Influenza A positive	Unknown
Acute otitis media, mastoiditis, meningitis	F	76	6	13	2	no	Oxygen therapy	Recurrent pulmonary embolism, cataract surgery	No	Cerebro-spinal fluid culture, blood culture	3	Not tested	1 day
Pneumonia	F	64	5	13	3	no	Noninvasive mechanical ventilation	COPD Gold 3, depression	No	Blood culture	1	Not tested	4 days
Pneumonia	M	66	6	21	8	yes	Invasive mechanical ventilation, vasopres-sors, CRRT, ECMO, massive transfusion	Type 2 diabetes mellitus	No	Blood culture, urine antigen test, sputum	3	Negative	3 days
Pneumonia	M	58	3	12	15	yes	Invasive mechanical ventilation, vasopressors, CRRT	Obesity, type 2 diabetes mellitus, basal cell carcinome, amyloidosis, atrial flutter, nefrotic syndrome	Bortezomib/ dexamethason	Urine antigen test	Unknown	Negative	1.5 week
Meningitis, endocarditis (Case 4)	F	67	7	23	7	no	Invasive mechanical ventilation, vasopressors	Hyper Hypertension, hypercholes-terolemia, type 2 diabetes mellitus	No	Cerebro-spinal fluid culture, blood culture	Unknown	Not tested	1 week

COPD = chronic obstructive pulmonary disease; CRRT = continuous renal replacement therapy; ECMO = extra corporeal membrane oxygenation; h = hour; ICU = intensive care unit; MOF = multiple organ failure; SOFA = sequential organ failure assessment

pneumococcal infections during the 2017-2018 autumn and winter months in a tertiary care ICU.

In our cohort, almost all serotyped isolates were non-PCV10 serotypes, which corresponds with replacement of infections by non-vaccine serotype pneumococci and matches IPD surveillance in Europe and the Netherlands, reporting 86% to 90% non-PCV10 serotype IPD, with major serotypes 8 and 3.[4,19]

With serotypes, clinical presentation may change over time. We admitted a notable and unusually high number

of severe pneumococcal infections, and comparison of our data with a neighbouring ICU suggests an increase in severe and atypical presentations over time. We report a relatively high mortality rate of 47% in 2017-2018 compared to preceding Dutch IPD cohorts; 28% in the secondary care ICU population in 2001-2016, and 13% in the general adult population 2008-2012.[14,22] IPD mortality rates in an ICU setting were 14 to 29% for pneumonia cases, and 25% for meningitis cases.[23-25] The sole study reporting on a comparable IPD case mix in ICU stems from 1983, where mortality was 76%.[26] In our cohort, three serotyped deadly infections concerned non-vaccine serotype 8, which, since 2013,[27] is the most common serotype in the Netherlands and more recently throughout Europe.[28]

Two previous studies reported that the rise in non-vaccine serotypes predominantly affected immunocompromised hosts, in addition to the elderly.[13,29] Most patients were over 65 years old in our cohort, but several patients were younger. Also, patients with few risk factors contracted severe pneumococcal disease. As it is sometimes a previously unknown risk factor, it may be worthwhile to screen for immunodeficiency in these cases.

In the group of typical presentations, most patients presented with pneumonia; mortality (4/9) seemed somewhat higher than the 24% in the preceding neighbouring ICU cohort or the 14-29% reported elsewhere previously.[23-25] We had two patients with pneumococcal meningitis, of whom, one patient died after a short course of disease.

Pneumococcal pericarditis is an unusual but severe complication of pneumococcal infection with high mortality rates.[30-32] In one multinational prospective study, cardiac complications were reported in 1% of 844 patients with *S. pneumoniae* bacteraemia carrying a mortality rate of 25%. In our cohort, 20% of patients developed cardiac complications with a mortality rate of 33%. Interestingly, recent reports found evidence of invasion of *S. pneumoniae* into the myocardium in animal models, which disrupts cardiac muscle function leading to arrhythmias and heart failure.[33,34]

Pneumococcal peritonitis is also a rare manifestation of pneumococcal infection. Spontaneous (primary) pneumococcal peritonitis occurs in patients with underlying liver cirrhosis and is reported in association with respiratory tract infections.[35-36] Secondary peritonitis is described with appendicitis or with genitourinary tract infection related to, for example, intrauterine tract devices.[35] In our cohort, one patient had pneumococcal peritonitis after an endoscopic procedure. Her medical file did not mention any respiratory symptoms. In immunodeficient patients, secondary pneumococcal infection has been described after endoscopy or variceal bleeding.

Regarding the diagnosis of pneumococcal disease, 11 of our cases cultures (blood, ascites, CSF, sputum) were positive for pneumococci. In seven cases, non-culture-based tests were found positive (PAT, PCR). Moreover, in four cases, these tests were not supported by a concomitant positive culture. This emphasises the increasingly important role of PAT and PCR testing in diagnosing pneumococcal disease.

A major limitation to our study is the retrospective single-centre design. For data collection, we depended on the accuracy of the electronic patient file.

As our results hint at a change in the severity of presentation of pneumococcal infections, a prospective systematic clinical registry for severe (invasive) pneumococcal infections might help to clarify trends in disease manifestations, serotype distributions, risk factors, and outcomes.

CONCLUSION

We report a case series of pneumococcal infections in adults with extreme disease manifestations. While our data are not representative enough to suggest a trend in increasing disease severity, interesting differences are seen when compared to a historical cohort from a neighbouring hospital. Given ongoing changes in infecting serotypes, a systematic clinical registry of pneumococcal infections in the ICU may be a valuable addition to pneumococcal disease surveillance.

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DISCLOSURE

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SPECIAL REPORT

Monoclonal gammopathy with significance: case series and literature review

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ABSTRACT

Monoclonal gammopathy of undetermined significance (MGUS) is considered an asymptomatic precursor of malignant lymphoid disorders. This case series and literature review shows that these monoclonal gammopathies can cause significant morbidity. We describe a patient with angioedema due to acquired C1-esterase inhibitor deficiency, a patient with cryoglobulinemia type II causing skin vasculitis and glomerulonephritis, and a patient with glomerulonephritis and nephrotic syndrome – all caused by a monoclonal gammopathy that can be classified as MGUS. Clinicians should be familiar with these consequences of monoclonal gammopathies. The term MGUS should only be used in patients without organ damage caused by monoclonal gammopathies.

KEYWORDS

Angioedema, cryoglobulinemia, MGRS, MGUS, monoclonal gammopathy

INTRODUCTION

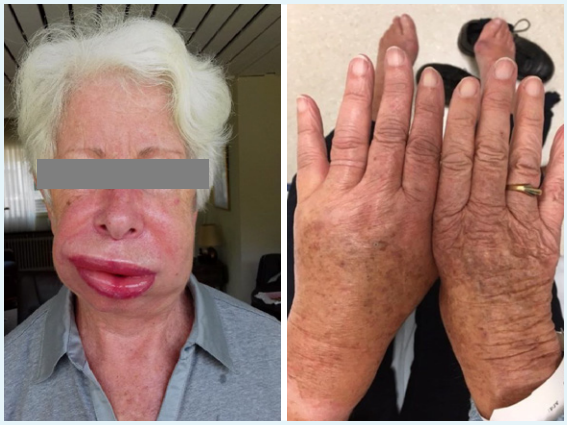
Monoclonal gammopathy of undetermined significance (MGUS) is considered an asymptomatic precursor of malignant lymphoid disorders. In MGUS, an abnormal clone of a single plasma cell (precursor) produces a monoclonal immunoglobulin (M-protein) and/or immunoglobulin light chains. The four diagnostic criteria for MGUS according to the International Myeloma Working Group are (1) M-protein lower than 30 g/l, (2) bone marrow plasma cells < 10% and low level of plasma cell infiltration in a trephine biopsy (if done), (3) no evidence of other B-cell proliferative disorders, and (4) no related organ or tissue impairment (CRAB-criteria: hypercalcaemia, renal insufficiency, anaemia, bone lesions).[1]

The prevalence of MGUS is 3.2% in people over the age of 50 years and 6.6% in people over the age of 80 years.[2] The finding of MGUS is often unrelated to the patient's primary medical problem. In general, patients with MGUS are not treated. However, despite the non-malignant nature of an 'MGUS-like' monoclonal gammopathy, it can cause significant morbidity. The next three cases will illustrate that MGUS can have severe consequences.

Case 1: Acquired C1-esterase inhibitor deficiency

A 78-year-old woman without relevant medical history was referred to the emergency department because of progressive unilateral oedema of the face, lips, and uvula since several hours (figure 1A). In the preceding days, she had had episodes of swelling of extremities (figure 1B). There was no fever, rash, or pruritus and the patient did not use medication. The patient was admitted to

Figure 1. *A) Oedema of (predominantly) right side of face; B) oedema of left hand*



A B

the Intensive Care Unit for observation. The swelling gradually disappeared after several hours. In the following three months, the patient presented almost every week to the emergency department because of similar episodes with unilateral swelling of the face, lips, and/or extremities. Laboratory investigations (table 1) revealed low complement C4 and C1-esterase inhibitor deficiency. Antibodies against C1-esterase inhibitor were not detected. In addition, there was a presence of M-protein IgG-lambda and monoclonal free light chain lambda. A positron emission tomography-computed tomography (PET/CT) scan showed no lytic ossal lesions, and bone marrow biopsy immunophenotyping showed 5% monoclonal plasma cells. We diagnosed the patient with angioedema due to acquired C1-oesterase inhibitor deficiency secondary to a monoclonal gammopathy.

Attacks of angioedema were treated with C1-esterase inhibitor when there was risk of a compromised airway. Treatment with tranexamic acid 1000 mg had no effect on frequency and severity of angioedema episodes. Instead, treatment with danazol 200 mg, two times a day was started, after which, the patient had no more episodes of angioedema in the next 11 months. The dosage of danazol was gradually decreased to 100 mg once daily.

Seven months after the start of danazol, M-protein concentration had increased to 33 g/l and renewed bone

Table 1. *Laboratory results*

	Reference	Case 1 (angioedema)	Case 2 (cryoglobulinemia)	Case 3 (MGRS)
Serum				
Haemoglobin (mmol/l)	M: 8.5 – 11.0 F: 7.5 – 10.0	8.0	6.9	6.9
Creatinine (µmol/l)	64 – 104	64	292	149
CKD-EPI eGFR (ml/min/1.73 m^2)	> 90	81	16	36
Albumin (g/l)	35 – 50	28		
Calcium (mmol/l)	2.15 – 2.60	2.21		
Ionised calcium (mmol/l)	1.12 – 1.32		1.13	1.25
M-protein (g/l)	Not present	26 (IgG-lambda)	Not present	14 (IgG-kappa)
Kappa-free light chain (mg/l)	6.7 – 22.4	22	170	750
Lambda-free light chain (mg/l)	8.3 – 27.0	180	55	28
Kappa/lambda-free light chain ratio	0.31 – 1.56	0.12	3.1	27
Cryoglobulins	Not present	Not present	Present*	Not present
C3 (g/l)	0.8 – 1.6	0.7	0.5	1.1
C4 (g/l)	0.14 – 0.45	< 0.02	< 0.02	0.14
C1q (IE/ml)	81 - 128	176		
C1-esterase inhibitor (E/ml)	0.63 – 1.82	0.39		
Urine				
Protein (g/24u)	< 0.20		8.20	3.60
Bence Jones (mg/24u)	Not present	Lambda light chain: 70	Not present	Kappa light chain
Erythrocytes (per visual field)	≤ 2	20 – 50**	3 – 10**	0 – 2
Erythrocyte cylinders (per visual field)	0	1 – 2	0	0

* Monoclonal IgM kappa 50 mg/l; polyclonal IgG 29 mg/l and IgA 11 mg/l
** With < 5% dysmorphia
C1q = complement component 1q; C3 = complement component 3; C4 = complement component 4; CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration; eGFR = estimated glomerular filtration rate; F = female; IgG = immunoglobulin G; M = male; M-protein = monoclonal immunoglobulin; MGRS = monoclonal gammopathy of renal significance.

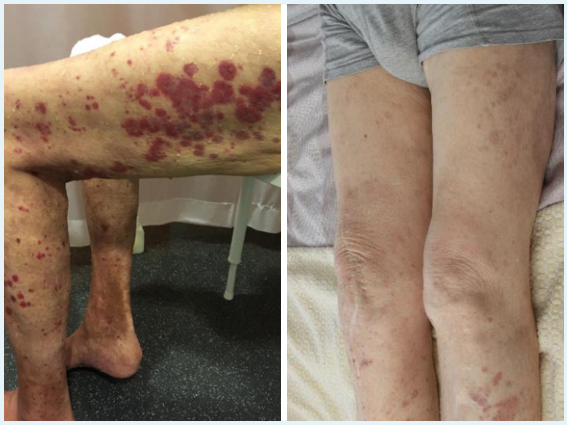
marrow biopsy showed 23% plasma cells. Moreover, Bence Jone protein was detected and urinary sediment showed erythrocyte cylinders (table 1). The patient was diagnosed with high-risk smouldering myeloma (Revised International Staging System, R-ISS stage II). Treatment with lenalidomide and dexamethasone (cycles of 28 days with lenalidomide from days 1 to 21 and dexamethasone on days 1, 8, 15, and 22) and pamidronic acid (once per four weeks) was started. Eight weeks after start of treatment (i.e., two cycles), M-protein concentration had decreased from 33 to 8 g/l, and danazol was ceased. Another eight weeks later (after four cycles), M-protein concentration was < 2 g/l and C1-esterase inhibitor levels had normalised. Until present day (six months after start of treatment), the patient did not have an attack of angioedema and serum M-protein remains undetectable.

Case 2: Cryoglobulinemia

An 83-year-old man with a history of myocardial infarction (1989), peripheral vascular disease and femoral-popliteal bypass (2018) was referred to the nephrology outpatient department with progressive renal insufficiency and nephrotic-range proteinuria (8.20 g/24hr). Within seven months, the estimated glomerular filtration rate (eGFR) had gradually decreased from 64 to 16 ml/min/1.73 m^2. Furthermore, the patient had skin lesions that had started as episodic purpura on the legs seven months earlier, but had progressed to permanent non-pruritic palpable conflating purpura on the trunk and all extremities (figure 2A). Laboratory investigations (table 1) revealed monoclonal free light chain type kappa, presence of cryoglobulins (monoclonal IgM kappa and polyclonal IgG and IgA), and low complement C3 and C4. Unfortunately, rheumatoid factor was not determined. Hepatitis B and C serology were negative. There were no symptoms or findings suggesting systemic disease such as Sjögren syndrome. A kidney biopsy showed glomerulonephritis with deposition of IgM, kappa, and C3, and a skin biopsy showed leucocytoclastic vasculitis with deposition of C3 in the vascular wall. Bone marrow examination revealed 1% monoclonal B cells, without increased percentage of plasma cells or lymphocytes. We diagnosed the patient with cryoglobulinemia type II with skin vasculitis and glomerulonephritis secondary to a monoclonal gammopathy. We started treatment with prednisone 60 mg per day. This had a spectacular response. After only 2.5 weeks, the skin lesions had almost vanished (figure 2B) and the eGFR had improved from 16 to 45 ml/min/1.73 m^2. Furthermore, cryoglobulins were no longer detectable and the kappa/lambda light chain ratio had normalised. Because of this quick and complete response, and frailty of the patient, we decided to withhold targeted treatment of the monoclonal B cells with rituximab.

In the following seven months, the prednisone was slowly tapered to a total daily dose of 15 mg. At that point, kappa/lambda light chain ratio increased to 2.0 (kappa free light chain 45 mg/l), cryoglobulins were detectable again, and proteinuria increased somewhat (urine albumin/creatinine ratio 102 mg/mmol). Despite these signs of relapse, we decided to continue monotherapy with prednisone 15 mg, because of stable kidney function, absence of skin lesions, and increased frailty in the meantime (amputation of the upper leg due to acute ischemia and de novo chronic obstructive pulmonary disease). The patient died several weeks later due to this comorbidity.

Figure 2. *Skin vasculitis, A) before treatment, B) 2.5 weeks after start of prednisone*



A B

Case 3: Monoclonal gammopathy of renal significance

An 86-year-old man with a history of hypertension and stable chronic kidney disease was referred to the nephrologist because of progressive decrease in kidney function (increase of serum creatinine from 115 to 144 µmol/l in one year). Physical examination was normal except for a blood pressure of 160/80 mmHg despite use of losartan 100 mg daily. Laboratory results (table 1) showed progressive kidney failure with nephrotic-range proteinuria and presence of monoclonal IgG kappa and free light chain kappa. Bone marrow biopsy showed 6% plasma cells. PET-CT revealed no osteolytic lesions. A renal biopsy showed mesangiocapillary glomerulopathy with signs of thrombotic microangiopathy and tubulopathy secondary to paraproteinemia, and 40% sclerosed glomeruli and intima fibrosis of arterial branches. We diagnosed the patient with glomerulonephritis with nephrotic syndrome caused by monoclonal gammopathy of renal significance (MGRS). Because of high age, lack of symptomatology, and relatively mild expression, we decided on a wait-and-see policy.

Two years later, the patient complained of back pain. A CT scan revealed multiple osteolytic lesions in skull and spine.

A renewed bone marrow biopsy again showed 5-10% plasma cells (the same as two years earlier). Serum creatinine had increased to 244 µmol/l (Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) for eGFR 20 ml/min/1.73 m^2). The quantity of M-protein, kappa/lambda free light chain ratio, and urine albumin/creatinine ratio were lower than two years earlier (12 g/l, 21, and 262 mg/mmol, respectively). Although plasma cells in the bone marrow were only 5-10%, patient was treated as having symptomatic multiple myeloma. Treatment with dexamethasone 20 mg per week, lenalidomide 5 mg per day, and pamidronic acid 30 mg once per month was started. After three months, M-protein had decreased to 8 g/l and kappa/lambda free light chain ratio to 7.5. However, serum creatinine and proteinuria had not improved and patient had developed a pancytopenia. As improvement of renal function was not expected with continuing therapy, and whereas side effects as pancytopenia would worsen, it was decided to cease treatment. In the six months thereafter, clinical condition and renal function gradually decreased (serum creatinine 339 µmol/l/1.73 m^2, CKD-EPI eGFR 13 ml/min/1.73 m^2), while kappa/lambda free light chain ratio rose to 19. Because of immobility and poor prognosis, the patient wished to transfer care to his general practitioner for palliative care.

DISCUSSION

We describe three patients with a monoclonal gammopathy that can be classified as monoclonal gammopathy of undetermined significance (MGUS). However, the diagnosis MGUS is not applicable, as the significance of the monoclonal gammopathy in these patients is not 'undetermined', but pathologic.

Acquired C1-esterase inhibitor deficiency (case 1)

Patient 1 was diagnosed with acquired angioedema caused by C1-esterase inhibitor deficiency (AAE). AAE is rare and manifests as recurrent subcutaneous non-pruritic swelling of the face, larynx, tongue, extremities, abdominal wall, and/or genital organs, without urticaria.[3] An attack typically lasts for 24 to 72 hours.[3] Angioedema of the tongue and/or larynx can be life-threatening.[3] The median age at first presentation is 57-62 years.[3,4] In most patients with AAE, C4, C1-esterase inhibitor and C1q levels, and C1-esterase inhibitor activity are reduced.[5,6] A majority (75-83%) of patients with AAE also have a lymphoproliferative disorder, and 26-57% of patients with AAE have an 'MGUS-like' monoclonal gammopathy.[3,4,7] There are two hypotheses on the association between C1-estarase inhibitor deficiency and these lymphoproliferative disorders: (1) the produced monoclonal immunoglobulin has anti-C1-esterase-inhibitor-activity, and (2) excessive activation of C1 by immune complexes containing the monoclonal immunoglobulin results in consumption of C1 inhibitor.[8] If detectable, the anti-C1-esterase inhibitor antibodies frequently exhibit the same isotype as the monoclonal immunoglobulin.[7]

Acute attacks of angioedema can be treated with tranexamic acid (inhibition of plasmin formation; only for moderate attacks), icatibant (bradykinin receptor antagonist) and/or C1-esterase inhibitor concentrate.[3] Tranexamic acid and danazol (an androgenic steroid that stimulates hepatic production of C1-esterase inhibitor) are effective in reducing the frequency of angioedema attacks, in 76-93% and 33-75% of patients, respectively.[3,4] Moreover, based on limited data, administration of rituximab and treatment of the underlying disease seem also effective in reducing the frequency of attacks.[3,7,9] In five of the six patients with MGUS, treatment with rituximab resulted in response (defined as no attacks or > 50% reduction in attacks over the next six months).[3]

Monoclonal gammopathy of renal significance (cases 2 and 3)

Patients 2 and 3 were diagnosed with monoclonal gammopathy of renal significance (MGRS).

The term MGRS was introduced in 2012 by the International Kidney and Monoclonal Gammopathy Research Group.[10] The diagnostic criteria of MGRS are (1) B-cell or plasma cell clonal lymphoproliferation that does not cause tumour complications or meet any current haematological criteria for specific therapy, and (2) kidney biopsy with presence of one or more lesions that are related to the produced monoclonal immunoglobulin.[11] MGRS is an umbrella term for different types of kidney damage, mainly caused by deposition of (fragments of) monoclonal immunoglobulins. These different types of MGRS are categorised by localisation of lesions in the nephron and their pathological features (fibrillar, microtubular, inclusion/crystalline deposits, non-organized monoclonal immunoglobulin deposits, or absence of monoclonal immunoglobulin deposits).[11] In general, MGRS should be suspected, and a kidney biopsy should be performed in patients with a monoclonal gammopathy and unexplained kidney disease, in patients with known risk factors for chronic kidney disease but an atypical clinical course, and in patients with kidney disease and monoclonal gammopathy aged < 50 years.[11]

In general, the overall survival of patients with MGRS is significantly better than multiple myeloma, but the renal outcomes are not. Moreover, if left untreated, the recurrence rate after kidney transplantation is very high (> 80%).[10,11] Thus, treatment of MGRS is indicated in order to reduce mortality, prevent kidney failure, improve kidney function and, in patients with end-stage renal disease, prevent recurrence after transplantation. The treatment goal of MGRS is complete haematologic remission by

targeting the underlying B-cell clone by chemotherapy or an autologous stem cell transplantation.[12]

Cryoglobulinemia type II (case 2)

The patient of case 2 was diagnosed with cryoglobulinemia type II with skin vasculitis and glomerulonephritis secondary to a monoclonal gammopathy. Cryoglobulinemia is defined by the presence of cryoglobulins, immunoglobulins that precipitate at a temperature below 37 ^{0}C, in the circulation. Brouet et al. defined three types of cryoglobulinemia in 1974.[13] Only type I and type II cryoglobulinemia are associated with monoclonal gammopathies.

The cryoglobulins in type II cryoglobulinemia are immune complexes of a monoclonal immunoglobulin (usually IgM kappa) with antibody activity against IgG and polyclonal IgG.[13] As in our patient, serum complement levels are usually decreased in type II cryoglobulinemia.[14] Most cases (82%) of type II cryoglobulinemia are associated with a chronic hepatitis C virus infection.[14] Other associated conditions are monoclonal gammopathies, haematological malignancies, hepatitis B virus infection, HIV infection, and autoimmune diseases, mainly Sjögren syndrome.[14,15] Slightly more than half (53-60%) of patients with type II cryoglobulinemia, including our patient, have vascular purpura, often triggered by standing or exercise.[13,14] Other symptoms are Raynaud's phenomenon, arthralgia and arthritis, as well as peripheral neuropathy and kidney damage (prevalence of the latter is 33-35%).[13,14] Type II cryoglobulinemia can also be asymptomatic.[14]

Treatment is not always indicated. In patients without symptoms or with only few systemic symptoms and/or only episodic purpuric flares, observation alone is recommended.[12] An underlying condition, such as hepatitis C virus infection, should always be treated. Indications for treatment are the presence of vasculitis, progressive systemic symptoms, and MGRS.[12] Monotherapy with high-dose corticosteroids is very effective in reducing symptoms of vasculitis. Treatment with rituximab is indicated in patients with recurrent symptoms or renal involvement.[12] Next, chemotherapy (rituximab-containing regimen or bendamustine) should be considered in patients with Waldenström macroglobulinemia or B-cell lymphoma with symptoms more significant than occasional purpura.[12] In patients with acute severe systemic symptoms and/or severe organ involvement plasma exchange should be considered.[12]

CONCLUSION

We have described three cases of an 'MGUS-like' monoclonal gammopathy causing significant morbidity. Assessment for presence of monoclonal gammopathy is highly recommended in patients with unexplained kidney damage (MGRS), acquired angioedema due to C1-esterase inhibitor deficiency, and/or unexplained purpura. Asymptomatic monoclonal gammopathy (MGUS) must be distinguished from monoclonal gammopathies with significance, as prognosis and management are vastly different.

DISCLOSURE

There was no funding for this study. There are no conflicts of interest.

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CASE REPORT

HIV-associated and idiopathic-acquired haemophilia A: A single-centre case series from Cape Town, South Africa

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ABSTRACT

Acquired haemophilia A is a rare coagulation disorder, which can lead to life-threatening haemorrhages if not identified and treated promptly. It is characterised by the presence of autoantibodies (inhibitors) to factor VIII. Acquired haemophilia A associated with HIV is a rare but well described phenomenon with limited directions to its management. We comparatively describe four patients – two with HIV and two without - that presented with unusual bleeding episodes with a prolonged activated partial thromboplastin time secondary to factor VIII inhibitors. An empiric observation is that the patients with acquired haemophilia A associated with HIV had higher antibody titres at presentation, that required more prolonged immunosuppressive therapy to induce remission.

KEYWORDS

Acquired haemophilia, HIV

What was known on this topic?
Acquired haemophilia (AHA) is a rare bleeding disorder caused by the formation of factor VIII (FVIII) inhibitors, which are autoantibodies against FVIII. If left untreated, the condition can lead to life-threatening haemorrhage. About half of all cases are idiopathic. The remaining cases are associated with autoimmune diseases, pregnancy, malignancies, infections, and certain medications. Several case reports of HIV-associated AHA have been published.

What does this add?
The HIV-associated AHA patients in this sample presented with a higher antibody titre and required a longer time on treatment to induce remission, compared with the idiopathic AHA patients. This observation correlates with other reports of HIV-associated AHA in the literature. Due to the rarity of this condition, recording, and registration of cases is of great importance, to improve understanding and future patient care.

INTRODUCTION

Acquired haemophilia A (AHA) is a rare coagulation disorder with a reported incidence of 1.20-1.48 million/year.[1,2] The condition is characterised by the production of autoantibodies to factor VIII (FVIII), leading to inhibition of FVIII. Early detection is essential, as most patients develop severe haemorrhage leading to considerable morbidity and mortality.[3]

The most commonly associated conditions are other autoimmune diseases, malignancies, pregnancy, specific pathogens (e.g., hepatitis B and C, human herpes virus-8), and drug exposure (including antibiotics, clopidogrel, and interferon), although in approximately 50% of patients, the appearance of these autoantibodies is idiopathic.[3,4]

Acquired FVIII inhibitors associated with human immunodeficiency virus (HIV) infection, either with or without hepatitis C virus (HCV) co-infection, have been described by several case reports in the literature.[5-10] South Africa has the largest HIV epidemic in the world, with a prevalence of 18.9%.[11] The rare association of AHA with HIV infection is therefore relatively more prevalent in

South African patients and experience in how to treat them, essential.

We present here a case series of the two HIV-positive patients, as well as the two HIV-negative patients, with AHA diagnosed and treated by the haematology unit at Groote Schuur Hospital in Cape Town, South Africa, from 2011-2019.

CASES

We suspected a diagnosis of AHA in all patients when a prolonged activated partial thromboplastin time (aPTT), which did not correct with mixing studies, was observed. Subsequently, we found decreased FVIII activity and presence of FVIII inhibitors, confirming the diagnosis. Platelet count and prothrombin time (PT) were characteristically normal in all patients.

There is no formalised treatment protocol for AHA in South Africa and management is determined by local expertise, dependent on both availability and cost of drugs. Once haemostasis is achieved, the focus of therapy is inhibitor eradication. In our setting we endeavour to achieve immunosuppression with corticosteroids and an alkylator agent such as cyclophosphamide. As the public health sector in South Africa does have significant resource constraints, and administration of biologic immune modulators (e.g., rituximab), as well as bypassing agents, are generally reserved for refractory cases, due to their high cost. See table 1 for an overview of all patients.

HIV-positive patients

Patient 1

Patient 1 was a 43-year-old female, diagnosed with HIV and started on antiretroviral drugs (ARVs; stavudine, lamivudine, and efavirenz) two years before presentation. She was also receiving rifampicin, ethionamide, ethambutol, and pyrazinamide for isoniazid-resistant pulmonary tuberculosis, diagnosed two months prior to presenting. At presentation, her CD4 count was 118 cells/µl and HIV viral load (VL) was undetectable.

She presented with bilateral knee haemarthrosis and easy bruising with a duration of two weeks. Initial laboratory investigations showed aPTT of 140.1 seconds (s), which did not correct on mixing studies, and haemoglobin (Hb) of 3 g/dl. Subsequently, we observed a FVIII inhibitor titre of 218 BU/ml and FVIII levels of 0 IU/dl. Testing for

Table 1. *Patient characteristics and outcomes*

Patient	Age/ Sex	Year of Dx	Associated illness	Symptoms	aPTT (seconds)	Minimum Hb (g/dl)	FVIII (IU/dl)	FVIII Inhibitor (BU/ml) at diagnosis	Bypassing agent needed (FEIBA)	Treatment	Time to absence of FVIII Inhibitor (months from starting Rx)
1	43/F	2011	HIV	Knee haemarthrosis, easy bruising	140.1	3	0	218	No	Steroids, cyclophosphamide, chlorambucil	9 (off all treatment after 15 months)
2	45/F	2015	HIV/ IRIS	Haematuria, muscle haematomas, easy bruising	150.3	5.9	0	629.5 (peaked at 1106)	Yes	Steroids, cyclophosphamide, rituximab	7 (off al treatment after 4.5 years)
3	50/M	2018	None	Neck haematoma, malaena stool	172.5	7.1	2.9	172.65	Yes	Steroids, cyclophosphamide	1 (off all treatment after 6 months)
4	84/F	2019	None	Neck haematoma	117.4	10.7	0.8	33.67	No	Steroids, cyclophosphamide	2 (currently on steroids and cyclophosphamide; 9 weeks on Rx)

aPPT = activated partial thromboplastin time; BU = Bethesda units; Dx = diagnosis; F = female; FEIBA = factor eight inhibitor bypassing activity; FVIII = factor VIII; HIV = human immunodeficiency virus; IRIS = immune reconstitution inflammatory syndrome; M = male; Rx = treatment

antinuclear antibody (ANA), anti-double stranded DNA antibodies (anti-dsDNA), lupus anticoagulant, and hepatitis B and C were all negative.

She received red cell transfusions and was given methylprednisolone intravenously (IV) at 500 mg/day for four days, which was then changed to oral prednisone at 1.5 mg/kg.

The patient experienced no further bleeds, but a month later was given cyclophosphamide (1g IV weekly x 2 doses) as her aPTT, although much improved, was still prolonged and she still had a high FVIII inhibitor titre (47.7s and 54.56 BU/ml, respectively).

One week later, her aPTT had increased again to 60 s; thus, chlorambucil at a dose of 6 mg daily was commenced.

Over the following months, corticosteroids were tapered and aPTT and FVIII inhibitor levels followed a downward trend. Nine months after commencing immunosuppressive therapy, inhibitors were absent and corticosteroids were stopped. All signs of bleeding, including the haemarthrosis, had fully resolved. Chlorambucil was continued for a year in total. The patient is seen for follow-up annually and has had no relapse to date – eight years after achieving remission.

Patient 2

Patient 2 was a 45-year-old female who had been diagnosed with HIV and started on ARVs (tenofovir. emtricitabine, and efavirenz) two weeks prior to presenting. Her CD4 count at presentation was 144 cells/µl and HIV VL was undetectable at presentation.

She presented with a several-day history of painless haematuria, easy bruising, and large haematomas to the lower limbs. Initial laboratory investigations showed aPTT of 150.3 s, which did not correct on mixing studies, and Hb of 5.9 g/dl. Subsequently, we observed a FVIII inhibitor titre of 629.5 BU/ml and FVIII levels of 0 IU/dl. ANA, anti-dsDNA, lupus anticoagulant and viral hepatitis studies were negative. We also identified tenofovir-induced nephropathy, which resolved when ARVs were changed to stavudine, lamivudine, and efavirenz.

Patient 2 received blood transfusions and one dose of methylprednisolone 125 mg IV, followed by prednisone at 1.5 mg/kg. Symptoms began resolving and aPTT improved until one week later, when her steroid dose was weaned to 1 mg/kg and she experienced a re-bleed and prolongation of aPPT to above 150 s. This resolved when the original steroid dose was resumed.

Patient 2 experienced a re-bleed into her thigh muscle requiring a blood transfusion six weeks later, and cyclophosphamide 100 mg daily was added to her treatment. At this point, the FVIII inhibitor level had increased to 1103.6 BU/ml. She also required two doses (50 U/kg each) of FEIBA (Factor Eight Inhibitor Bypassing Activity), due to failure of the haematoma to resolve.

One month later, another attempt to wean her steroid and cyclophosphamide doses induced bilateral buttock haematomas, requiring multiple blood transfusions and, over the course of four days, five doses of FEIBA (50 U/kg each). At this stage, due to failure to wean immunosuppressive therapy as well as the need for repeated costly blood transfusions and use of bypassing agent, rituximab was given (375 mg/m^2 per week for four doses). Several days after receiving the first dose of rituximab, the buttock haematoma worsened and she received eight more doses of FEIBA at 25-50 U/kg over the course of twelve days. The patient was discharged and an attempt was made to taper the steroid dose again.

The patient suffered one more re-bleed a month later, a thigh haematoma requiring one dose of FEIBA (50 U/kg). However, aPTT continued to improve and eventually, seven months after diagnosis and two months after rituximab administration, FVIII inhibitors were absent with aPTT and FVIII levels fully normalised.

Immunosuppressive therapy dosage was tapered and, four-and-a-half years after diagnosis, discontinued. FVIII inhibitors did recur asymptomatically at low titres (1.48 and 0.49 BU/ml) on two occasions: six months after receiving rituximab at 1.48 BU/ml (at the time, the patient was on prednisone 0.5 mg/kg and the inhibitors resolved when the dose was increased to 0.6 mg/kg for three weeks); and again 11 months after rituximab (the patient was on prednisone 0.5 mg/kg; this time the inhibitors resolved without any dose adjustment two weeks later).

HIV-negative patients

Patient 3

Patient 3 was a 50-year-old male taking enalapril, hydrochlorothiazide, and amlodipine for hypertension for more than 10 years.

He presented with a several-day history of a midline neck haematoma and melena stool. aPTT was found to be 172.5 s and Hb 7.1 g/dl. FVIII inhibitors were present at 172.5 BU/ml and FVIII levels were 2.9 IU/dl. ANA, anti-dsDNA antibodies, anti-cardiolipin antibodies, HIV, and hepatitis B and C testing were all negative. Lupus anticoagulant was weakly positive: patient/control ratio 1.28 (normal < 1.2).

The patient received FEIBA 50 U/kg daily for three days and the neck haematoma stabilised (the airway was never compromised) and melena resolved. Tranexamic acid was also given (1 g IV three times daily). He was started on prednisone 1.5 mg/kg and cyclophosphamide 100 mg daily. His aPTT decreased to 39 s the following week and he was discharged on the above medication. An absent inhibitor screen was found a month after initiating treatment and

his immunosuppressive medication was tapered over the following six months with no recurrence of symptoms.

Patient 4

Patient 4 was an 84-year-old female with a long history of hypertension, dyslipidaemia, chronic obstructive pulmonary disease (COPD), and hypothyroidism of unknown aetiology. Her chronic medication was amlodipine, furosemide, and simvastatin; inhaled salbutamol, budesonide, and levothyroxine.

She presented with a six-week history of a sub-lingual haematoma that had spread to the anterior neck, but was fortunately not compromising the airway. aPTT was 117.4 s and Hb 10.7 g/dl. FVIII inhibitors were present at 33.67 BU/ml and FVIII levels were 0.8 IU/dl. No other testing was done for autoimmunity.

She was started on methylprednisolone 80 mg IV daily and the haematoma soon began improving. This was changed to prednisone 1 mg/kg and cyclophosphamide 100 mg daily, one week later.

The haematoma resolved with no recurrence of symptoms and FVIII inhibitors were absent two months after initiating therapy. The patient is still on immunosuppression therapy nine weeks after being discharged, with dosage being tapered.

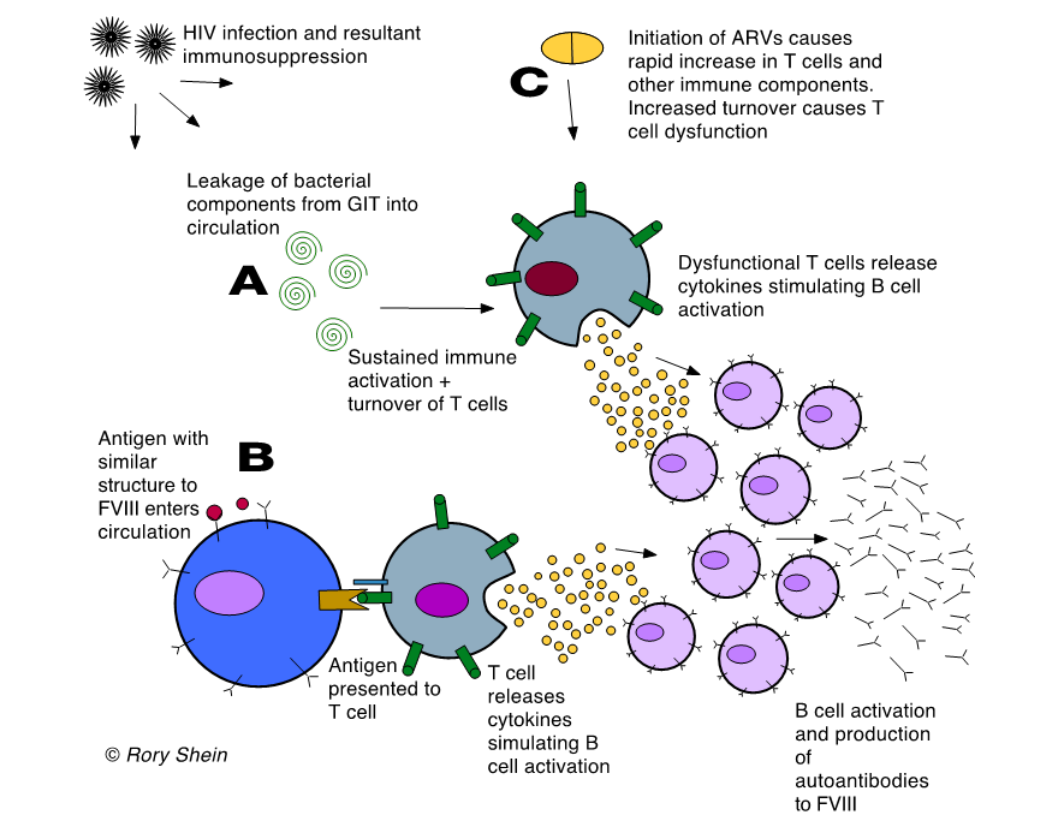
DISCUSSION

The precise mechanism for the appearance of FVIII inhibitors, usually a population of polyclonal IgG autoantibodies directed to FVIII[12], is not known.

In HIV, the breakdown of T-cell immune regulation and subsequent immune dysfunction allows for the development of autoimmunity, and numerous autoimmune conditions are associated with the disease. Various mechanisms of how this occurs have been postulated, including (figure 1):

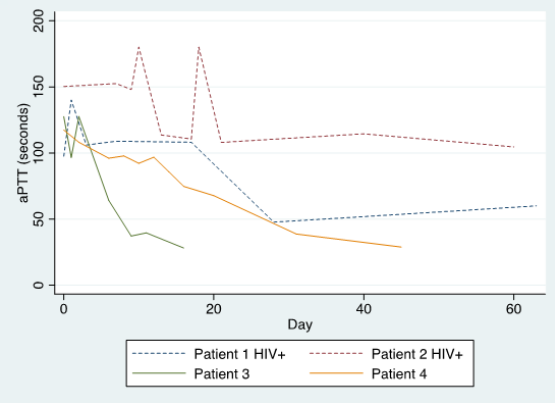
A. Long-term, sustained immune activation (due to leakage of bacterial components through the gut mucosa, caused by a dramatic decline in CD4+ T

Figure 1. *Schematic representation of possible mechanisms for the production of FVIII inhibitor in HIV (refer to text for explanation of pathways A, B and C)*



ARVs = antiretroviral drugs; FVIII = Factor VIII; GIT = Gastrointestinal Tract; HIV = human immunodeficiency virus

Figure 2. *aPTT v Time of the HIV-positive (Patient 1 and 2) and HIV-negative patients (Patient 3 and 4)*



aPTT = activated partial thromboplastin time;
HIV = human immunodeficiency virus

lymphocytes at this site seen early in HIV infection[13]), causing increased turnover and dysfunction of non-infected T cells. This leads to abnormal production of cytokines, causing the activation of B cells and increased production of immunoglobulins.[14]

B. Molecular mimicry of a self-antigen from an infectious agent, where T or B cells that react to pathogen-derived peptides cross react with host self-peptides.[9]
C. The immune dysfunction seen in patients with immune reconstitution inflammatory syndrome (IRIS) recently started on ARVs. This has been well described as a cause for autoimmune thyroid disease.[15,16]

The development of AHA in Patient 2 could have been due to IRIS, as she had started ARVs two weeks prior to developing FVIII inhibitors and had experienced a rapid decline in viral load during that time.

Most patients with AHA experience soft tissue, muscle, and mucosal (gastrointestinal tract, epistaxis, urinary tract)

Table 2. *Comparison of patients in this series with reported literature*

Study	Associated conditions/ medications	Factor VIII inhibitor titre at presentaion (BU/ml)	FVIII activity
A) HIV-positive AHA patients in present study compared with previously reported cases			
Present study Patient 1 (HIV+)	HIV on ARVs	218	0 IU/dl
Patient 2 (HIV+)		1106 (peak level)	0 IU/dl
Shweiber, et al. (2005)[5]	HIV, HCV. On ARVs, IFN-α	> 20	2%
Paul, et al (2007)[6]	HIV, HCV. On ARVs, IFN-α	800	NR
Migliore, et al. (2009)[7]	HIV, on ARVs	278	< 1 IU/dl
Zeichner, et al. (2013)[8]	HIV, HCV, not on ARVs	230.4	< 1% (80-150)
Rattanathammethee, et al. (2014)[9]	HIV on ARVs	140	0,7% (60-150)
Rivoisy, et al (2014)[10] Patient 1	HIV not on ARVs	11	< 1%
Patient 2		160	< 1%
B) Non-HIV-infected AHA patients in present study compared with two registry-based studies of AHA from all causes			
Present study Patient 3 (HIV-)	None	172.65	2.9 IU/dl
Patient 4 (HIV-)		33.67	0.8 IU/dl
Collins, et al. (2007)[2] n = 154	All causes	Median: 7.2 (Range: 1.4-219) *(fatal bleeds only)*	Median: 4 IU/dl (Range: 1-12) *(fatal bleeds only)*
Knoebel, et al. (2012)[4] n = 501	All causes	Median: 12.8 (IQR: 4.3-42.4)	Median: 2 U/dl (IQR: 1-5)

HCV = hepatitis C virus; HIV = human immunodeficiency virus; IFN = interferon; IQR =interquartile range; NR = not reported

bleeds, while bleeding into the joints is rare (as opposed to the bleeding pattern in congenital haemophilia).[3] Interestingly, this was not the case with Patient 1, whose primary sites of bleeding were both knee joints.

The HIV-positive patients in this series had a significantly higher FVIII inhibitor titre and lower FVIII level than the HIV-negative patients, and had a much longer and more complicated course of treatment to induce their remission (see figure 2). This reflects existing data that shows that higher inhibitor titre and lower FVIII at diagnosis are associated with a longer time to remission.[17] This correlates with other case reports of HIV-infected AHA patients, as well as with British and European multicentre registry-based studies of patients with AHA from all causes (see table 2). Inhibitors also developed at a younger age in the HIV-positive patients. All four patients in this case series survived and responded to immunosuppressive treatment.

As mentioned above, treatment of AHA patients in the SA public health sector is especially challenging due to resource constraints. Biologic immune modulators need special motivation and FEIBA is reserved for patients with non-resolving bleeds. As can be seen from Patient 2 above, the delay in administering rituximab led to slower resolution of symptoms and the need to use more FEIBA, ironically resulting in far more treatment costs than if it had been given sooner. This case series will serve to better inform the 'balancing-act' necessary to ensure efficient resource allocation while also optimising individual patient care.

CONCLUSION

AHA is a rare autoimmune condition, and in our setting, associated with HIV infection in two of four pts. Early detection, as well as prompt initiation of treatment is essential, and for one patient in our series, timid initial use of scarce resources may have led to higher eventual costs. All patients survived without permanent morbidity. In this small cohort, the association with HIV infection seemed to portend a longer pathway to remission. This observation also correlates with other cases of HIV-associated AHA reported in the literature. Due to the rarity of the disease, it is vital to record and register cases to improve understanding and optimise future patient care.

ACKNOWLEDGEMENT

We would like to express thanks to Sister Anne-Louise Cruikshank, haemophilia nurse co-ordinator of the Western Cape Province, South Africa, for identifying these patients and providing on-going management.

DISCLOSURE

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

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CASE REPORT

An unhealthy blush - secondary erythrocytosis due to waterpipe smoking

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ABSTRACT

Introduction: We present a case of a patient with a chronic carbon monoxide (CO) intoxication with facial plethora due to secondary erythrocytosis.

Case details: A 22-year-old male was referred by the dermatologist to our outpatient clinic for evaluation of polycythaemia. Laboratory results showed secondary erythrocytosis. After an extensive diagnostic evaluation, we diagnosed a chronic CO intoxication (carboxyhaemoglobin (COHb) level of 21%) without apparent complaints and facial plethora as the only clinical sign. The patient denied smoking tobacco or use of illicit drugs. On inspection of his house by the fire department, a *waterpipe was found in his bedroom*, which he used daily, according to his father. CO measurements in the house were normal. We treated the patient with high flow oxygen and advised him to quit smoking the waterpipe. Within a few weeks, the erythrocytosis normalised.

Discussion: We propose to test for the presence of an elevated COHb in all patients with a normal or high erythropoietin level. The test is not expensive and can easily be included as part of an examination, since CO intoxication has potentially disastrous consequences, and, as is illustrated with this case, chronic CO poisoning can be virtually asymptomatic. Not all individuals consider smoking a waterpipe the same as smoking or drugs, and therefore physicians need to specifically ask for its use.

KEYWORDS

Carbon monoxide poisoning, secondary polycythaemia, waterpipe

What was known on this topic?
Waterpipe smoking is increasingly popular in western countries, especially amongst the youth. It is known to cause acute CO intoxication with a variety of neurological and circulatory symptoms. The treatment of acute intoxication consists of administering high flow (normobaric) oxygen.

What does this add?
We present a case of a patient with a chronic CO intoxication with facial plethora due to secondary erythrocytosis as the only symptom. This case is different from other cases of CO intoxication because of the chronic nature of the intoxication and the lack of symptoms, except for facial plethora. In patients with an erythrocytosis and a normal or high EPO level, we propose to test for the presence of an elevated COHb even though there are no 'classic' signs of a CO intoxication.

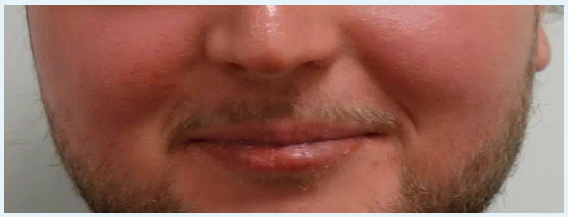
INTRODUCTION

Waterpipe smoking has become increasingly popular in Western countries, especially among young people.[1-3] It also poses emerging health risks like cardiovascular and respiratory disease, as well as a well-established risk of an acute CO intoxication. There are few reports on chronic use and consequences. We present an unusual case of a chronic CO intoxication due to waterpipe smoking.

Clinical presentation and clinical findings

A 22-year-old male was referred to our outpatient clinic by the dermatologist for evaluation of polycythaemia (haemoglobin 20.14 g/dl (12.5 mmol/l)). He was under

Figure 1. *Photo of the patients face showing facial plethora. This picture was published with full consent of the patient*



treatment by a dermatologist for 18 months for an invalidating facial plethora. The plethora started four years earlier without a significant contributing factor at the beginning. His last known normal haemoglobin level was from five years prior to the first consultation.

Diagnostic assessment

Our patient had no other complaints besides the facial plethora. Physical examination showed a striking facial erythema without telangiectasia (figure 1). He was slightly overweight (body mass index of 28 kg/m^2) but further physical examination was unremarkable. Peripheral oxygen saturation was 97%. There were no clinical findings suggesting a cardiopulmonary disease.

Laboratory analysis revealed a progressive elevation of the haemoglobin level (20.8 g/dl (12.9 mmol/l)). Haematocrit was 60% and white blood count, differential, and platelet counts were normal. Further analysis yielded an erythropoietin (EPO) level of 15.9 U/l (normal 4.4-15.8 U/l). The elevated EPO with a normal oxygen saturation was suspect for a secondary erythrocytosis, and we performed analysis for benign and malignant causes of elevated EPO. There were no abnormalities on chest X-ray, and abdominal ultrasound was unremarkable; in particular, there were no signs of renal cell carcinoma. A polysomnography showed no signs of obstructive sleep apnoea syndrome. A full-body computed tomography (CT) scan showed no signs of malignancy or other abnormalities. After these investigations, there still was no definite diagnosis. Despite the fact the erythrocytosis was chronic and the patient was not dyspnoeic, we performed an arterial blood gas analysis. The methaemoglobin level was normal, but the carboxyhaemoglobin (COHb) level was elevated (21%, normal < 5%), consistent with CO poisoning.

Therapeutic interventions

After diagnosing the CO poisoning, we confronted the patient with these findings. He still denied smoking cigarettes and using illicit drugs, but admitted smoking a waterpipe in a shisha lounge approximately two times per week. Despite the lack of other symptoms of a CO intoxication, we referred the patient to our emergency department for treatment with high flow oxygen therapy, which resulted in a lower COHb level of 6% within hours.

To identify the cause of the CO poisoning, we contacted the chemical, biological, radiological, and nuclear (CBRN) Tactical Advisor of the community health services to perform CO measurements at the residence of the patient. All CO values in the house were normal, even with active central heating. However, firemen found a waterpipe in the patient's bedroom. His father reported that the patient smoked it daily. We strongly recommended that the patient cease smoking the waterpipe, and within one month, his haemoglobin level dropped to 19.2 g/dl (11.9 mmol/l) and his EPO value normalised to 4.6 U/l. This confirmed the diagnosis of a facial plethora due to a secondary erythrocytosis as a result of chronic CO intoxication due to daily use of the waterpipe. The facial plethora also diminished.

DISCUSSION

Use of a waterpipe, also known as a 'hookah', 'shisha', or 'hubble bubble', has become increasingly popular amongst teenagers and young adults. In a study performed in the Netherlands, 6.9% of students between the ages of 12 and 16 reported use of a waterpipe in the previous month. In the adult population, there was a reported use of a waterpipe in 2.7% in the past 12 months[1] This worrisome trend seems to mainly affect Western countries. According to two larger studies recently published in the United Kingdom and the United States, there has been a recent increased prevalence of waterpipe smoking compared to cigarette smoking among high school and college students.[2,3]

One of the main reasons for its increased popularity is the social aspect of waterpipe smoking. Furthermore, it is perceived to have fewer health risks compared to cigarette smoking, but this is a misconception. People have high expectations of the filtering function of the water compartment in the system. However, multiple reports have been published revealing serious acute CO intoxications as a result of waterpipe usage.[4-6] In addition to CO intoxication, there are various short- and long-term complications due to waterpipe use. Examples are cardiovascular disease, decreased lung function, and an increased risk of malignancies. To our knowledge, secondary erythrocytosis without other symptoms of CO intoxication as a result of waterpipe usage has not been described before.

Secondary erythrocytosis

Polycythaemia is an abnormal elevation of haemoglobin as well as haematocrit in peripheral blood. Erythrocytosis refers to an elevated red blood cell (RBC) mass. This

Table 1. *Primary and secondary causes of erythrocytosis*

Primary erythrocytosis		
Congenital		
	Erythropoietin receptor mutations	
Acquired		
	Polycythemia vera	
Secondary erythrocytosis		
Congenital		
	Defects of the oxygen-sensing pathway	VHL gene mutations PHD2 mutations HIF-2α mutations
	Other congenital defects	Haemoglobin with high affinity for oxygen Bisphosphoglycerate mutase deficiency
Acquired		
	Central hypoxia	Chronic lung disease Right-left cardiopulmonary vascular - shunts Smokers erythrocytosis Hypoventilation syndromes, like sleep apnea Carbon monoxide poisoning Stay at high altitudes
	Local hypoxia	Renal artery stenosis Hydronephrosis Kidney failure Polycystic kidney disease Post-kidney transplantation
	Pathologic erythropoietin production	Cerebellar hemangioblastoma Meningioma Parathyroid pathology Renal cell carcinoma Hepatocellular carcinoma Pheochromocytoma Uterus leiomyoma
	Drugs	Erythropoietin administration Androgen administration

HIF-2α = hypoxia-inducible factors; PHD2 = prolyl hydroxylase domain; VHL = von Hippel Lindau

condition can be divided into congenital or acquired primary erythrocytosis and congenital or acquired secondary erythrocytosis. Table 1 shows the different causes of erythrocytosis.

Different guidelines and reviews provide a strategy to investigate the cause of the erythrocytosis, where clinical history and examination are essential to determine further investigations and management. Particular attention should be paid to signs related to potential secondary causes of erythrocytosis like cardiopulmonary diseases, EPO producing tumours, or smoking.[7-9] Tissue hypoxia is an important cause of secondary erythrocytosis.

In addition to full blood count analysis, arterial oxygen saturation is an easy test to identify tissue hypoxia. An oxygen saturation (SaO2) measurement of < 92% is associated with an absolute erythrocytosis.[10] However, there are situations where hypoxic erythrocytosis will exist despite a normal SaO2. Examples are high oxygen affinity haemoglobin, CO poisoning, and sleep apnoea syndrome. CO intoxication causes tissue hypoxia by replacing the oxygen molecules on haemoglobin.[11]

At the time of this case report, the local guidelines suggested to measure of COhb later in the diagnostic process. This recently changed, and COhb measurement is now incorporated early in the diagnostic process.

CONCLUSION

To our knowledge, this is one of the first reported cases of a relevant secondary erythrocytosis as a result of a chronic asymptomatic CO intoxication due to frequent waterpipe smoking.

In patients with a normal or high EPO level, we propose to test for the presence of an elevated COHb. The test is not expensive and may quickly reveal CO intoxication. As illustrated with this case, CO intoxication has potentially disastrous consequences, and patients with chronic CO poisoning may be virtually asymptomatic. Not all individuals consider using a waterpipe as dangerous as smoking or drugs, and therefore physicians need to specifically ask for its use during history.

DISCLOSURE

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

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PHOTO QUIZ

Sharp abdominal and scrotal pain

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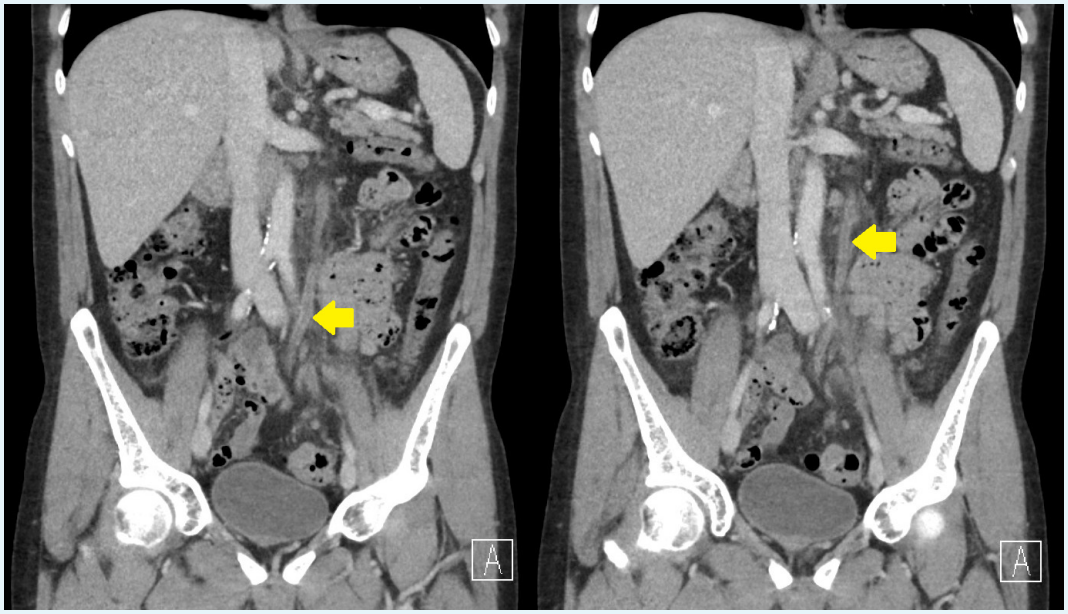
Figure 1. *Two coronal sections of the computed tomography scan of the abdomen*



The abnormality is identified with the yellow arrows.

CASE REPORT

A 58-year-old, previously healthy man presented to the emergency department. He complained of a progressive, paroxysmal sharp pain in the lower left abdomen accompanied by scrotal pain. Other symptoms were nausea, vomiting, non-bloody, watery stools and non-painful micturition with urge complaints.

We saw a haemodynamic stable, but painful patient without fever. Normal bowel sounds were heard upon abdominal auscultation. Palpation was painful, in particular, in the lower left abdomen. No abnormalities were found with scrotal examination.

Laboratory findings showed leucocytosis of 13.3x10^9/l (normal 4-10x10^9/l) and a C-reactive protein of 172 mg/l (normal 0-8 mg/l) in addition to diffuse liver chemistry abnormalities. Abdominal ultrasound showed thickening of the colon with fat infiltration of the mesenteric fat, especially in the lower left abdomen.

A computed tomography (CT) scan with intravenous contrast of the abdomen demonstrated diverticulitis of the sigmoid colon and the abnormality, as indicated in the images (figure 1).

WHAT IS YOUR DIAGNOSIS?

See page 207 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 206)

SHARP ABDOMINAL AND SCROTAL PAIN

DIAGNOSIS

The abnormality shown on the CT scan of the abdomen is a thrombosis of the left spermatic vein. Spermatic vein thrombosis is a rarely diagnosed pathology. Often, a spermatic vein thrombosis is an incidental finding upon a CT scan, or identified perioperatively with an incarcerated hernia as preoperative diagnosis.[1] Otherwise, patients present with testicular pain or scrotal swelling.[2]

A majority of thromboses are found in the left spermatic vein. Presumably, this is due to the same anatomical differences that cause more left-sided varicoceles. While the right spermatic vein enters the inferior vena cava directly, the left spermatic vein joins the renal vein, which enters the vena cava superior 8-10 cm higher than the right spermatic vein. This eventually leads to a higher pressure in the left spermatic vein and increased chance of stasis. Also, compression of the renal vein by the superior mesenteric artery and absent or incompetent valves are relatively common and contribute to venous stasis.[1,3]

Risk factors described are regional malignancies, tumours that affect the testicular venous drainage, varicocele, trauma, hypercoagulable states, systemic diseases, long-distant flights, certain drugs, inguinal hernia surgery, and vigorous exercise.[1,2,3,4]

There are no available guidelines for spermatic vein thrombosis treatment. Treatment recommendations therefore differ from conservative treatment with anticoagulants or anti-inflammatory drugs to surgically removing the thrombus.[2,3,4]

A case-control study conducted by Lenz et al. concluded that treatment with anticoagulation for spermatic vein thromboses seems to be indicated, given the recurrence venous thromboembolism rate for spermatic vein thromboses is similar to deep vein thrombosis controls.[3]

Our patient was treated for the spermatic vein thrombosis with apixaban for three months and recovered fully. The diverticulitis was treated conservatively with painkillers without complications. Because the diverticulitis was identified as the cause of the thrombosis, no further laboratory research to predisposing coagulopathy was carried out.

In conclusion, spermatic vein thrombosis is an uncommon diagnosed pathology which, in our opinion, seems to need treatment with anticoagulants.

DISCLOSURE

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

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PHOTO QUIZ

A tender erythematous facial plaque

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Figure 1. *A well-demarcated, tender, erythematous and anaesthetic plaque with polycyclic margin over left side of face. A cord-like thickened, greater auricular nerve over left side of neck was visible (black arrow).*

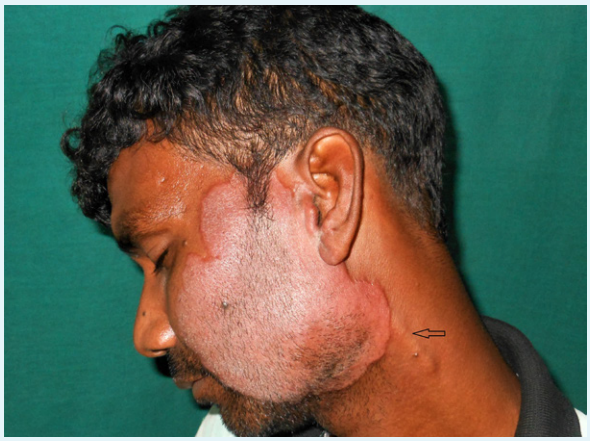
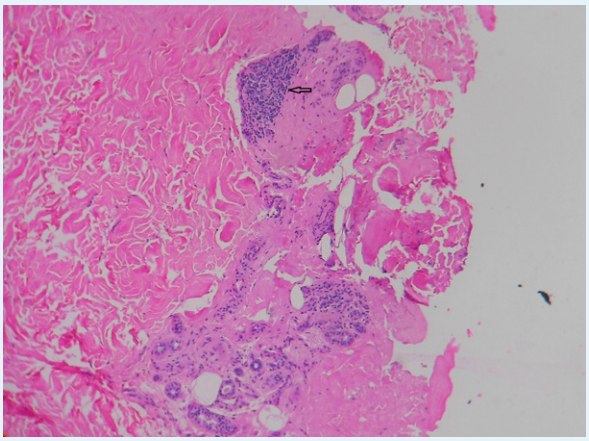


Figure 2. *Histopathological examination of the skin biopsy showed epithelioid granulomas (black arrow) arranged in a curvilinear pattern along the neurovascular bundle.*



CASE REPORT

A 26-year-old gentleman presented with a 3-month history of red-coloured rash over his face. The patient was treated with topical corticosteroid as a case of eczema, before presenting to us. He also complained of fever and joint paint in the preceding one month. Examination revealed a well-demarcated, tender, erythematous, and anaesthetic plaque with a polycyclic margin over the left side of his face. A cord-like, thickened, and tender greater auricular nerve over the left side of his neck was noted (figure 1). A thickened and tender ulnar nerve was documented on his left arm. Laboratory examination was notable for increased erythrocyte sedimentation rate (25 mm/hr, reference range 0-15 mm/hr). Histopathological examination of the erythematous margin revealed epithelioid granulomas arranged in a curvilinear pattern along the neurovascular bundle (figure 2). Fite-Faraco staining was negative.

WHAT IS YOUR DIAGNOSIS?

See page 209 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 208

A TENDER ERYTHEMATOUS FACIAL PLAQUE

DIAGNOSIS

Based on the clinical features and histopathological examination, we made a diagnosis of borderline tuberculoid leprosy with type 1 reaction. He received rifampicin (600 mg monthly), clofazimine (50 mg daily), dapsone (100 mg daily), and prednisolone (40 mg daily in a tapering dose) with significant improvement in 12 months.

Leprosy, or Hansen's disease, is an ancient bacterial disease caused by *Mycobacterium leprae* bacillus and continues to be a significant health problem in many parts of the world, especially in developing countries.[1] Owing to the long incubation period of *lepra* bacilli (1 to 30 years), a person may present with signs and symptoms of leprosy many years after leaving an endemic country, thus making it an imported disease into a non-endemic nation.

A case of leprosy is defined as the presence of one or more of the three cardinal signs: hypopigmented anaesthetic lesions, enlarged thickened peripheral nerves, and the presence of acid-fast bacilli. Leprosy produces a chronic infection in humans that primarily affects the peripheral nerves and skin, producing a spectrum of clinical phenotypes, based upon response of the host to the organism. According to clinical, bacteriological, histological, and immunological features, there are five types of leprosy: tuberculoid, borderline tuberculoid, mid-borderline, borderline lepromatous, and lepromatous.[2]

Type 1 reaction in leprosy occurs because of cellular hypersensitivity reaction and is characterised by acute inflammation in existing lesions. Involvement of peripheral nerves increase the likelihood of severe, rapid onset nerve damage and deformities.[3] In tuberculoid and borderline tuberculoid leprosy, a high degree of clinical suspicion is required to diagnose a case, since acid-fast bacilli within the context of leprosy are not always detected in slit skin smears and histopathological examination (with Fite-Faraco staining) of the lesions.

While encountering erythematous plaque over the face, a differential diagnosis of erysipelas (well-demarcated tender, erythematous plaques resulting from streptococcal infection of the dermis), cellulitis (ill-defined erythema, swelling, and tenderness resulting from infection of the deep dermis and subcutaneous tissue), arthropod-bite reaction (localised inflammatory reaction following an arthropod bite), tinea faciei (well-demarcated itchy, annular plaque with central clearing caused by fungal infection), and lymphocytoma cutis (type of cutaneous B-cell lymphoma) should be evaluated and ruled out. Early diagnosis and prompt treatment are of paramount importance to prevent nerve damage and resulting deformity and disability in the patient.

DISCLOSURE

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

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PHOTO QUIZ

Red streaks arising from the periumbilical area in a mirror-like pattern

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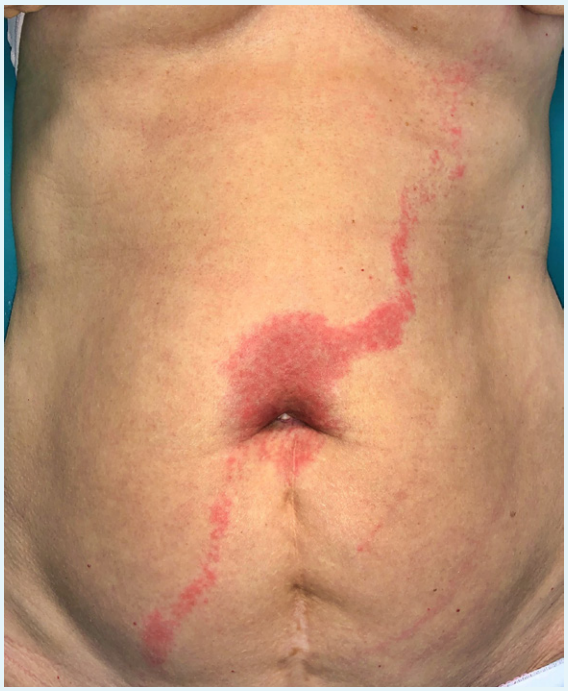
Figure 1. *Erythematoedematous linear streaks arising from the periumbilical area and extending in a mirror-like pattern*



CASE REPORT

A 50-year-old healthy woman with no prior dermatological history was referred to our department for evaluation of a 2-day history of burning erythematous plaques on the central part of her abdomen, arranged in an unusual shape. The patient had first noticed a red and intensely itchy papule in the umbilicus that was attributed to an arthropod bite, and within the first 24 hours, two linear skin lesions rapidly developed from the affected area. She denied any recent drug intake, travel history, local trauma, or contact with plants. Physical examination revealed an erythematous plaque around the umbilicus with well-defined borders. Examination also identified a central haemorrhagic punctum, associated with two erythematoedematous discontinuous linear streaks, arising from the periumbilical area and extending to the right groin and the left axilla in a mirror-like pattern (figure 1). No fever, lymph node enlargement, or other systemic symptoms were present and laboratory testing showed a normal blood cell count and no increasing of inflammatory parameters.

WHAT IS YOUR DIAGNOSIS?

See page 211 for the answer to this photo quiz

ANSWER TO PHOTO QUIZ (PAGE 210)

RED STREAKS ARISING FROM THE PERIUMBILICAL AREA IN A MIRROR-LIKE PATTERN

DIAGNOSIS

Based on the history and the examination findings, a diagnosis of non-infectious superficial lymphangitis induced by arthropod bite was made. Treatment with medium potency topical corticosteroids and oral antihistamines was started, achieving a complete response within four days.

Non-infectious superficial lymphangitis after an insect bite is an uncommon variant of acute lymphangitis without fever or lymphadenopathy, unlike classical streptococcal forms.[1,2]

It has been hypothesised that it is caused by an allergic reaction to toxins injected, with no evidence of local infection.[1] The toxins are drained by lymphatic vessels towards local lymph nodes, determining a linear inflammatory response in the overlying skin.[1,3] Certain subsets of patients, like those with haematological diseases, are prone to developing greater reactions to insect bites, including lymphangitic streaking.[2]

In order to exclude bacterial lymphangitis, an accurate differential diagnosis should be made that could benefit from an antibiotic course to avoid further complications. Other entities that may cause similar skin lesions include phytophotodermatitis, lymphatic filariasis, Mondor's disease, or superficial migratory thrombophlebitis.

Although non-complicated superficial lymphangitis resolves spontaneously after a few days as the hypersensitivity reaction diminishes, treatment with topical corticosteroids and oral antihistamines may improve the patient's symptoms.

In this case, the casual fact that the arthropod bite occurred in the umbilicus resulted in two different streaks, perfectly representing lymphatic drainage from central abdomen to both axillary and groin nodes in left and right sides, leading to a bizarre and unique clinical image.[4]

DISCLOSURES

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

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PHOTO QUIZ

Something is missing

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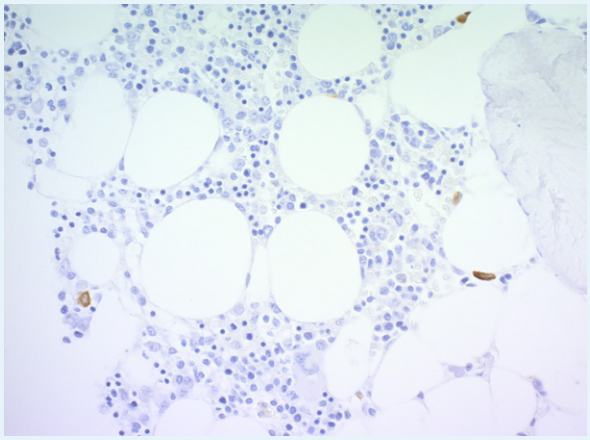
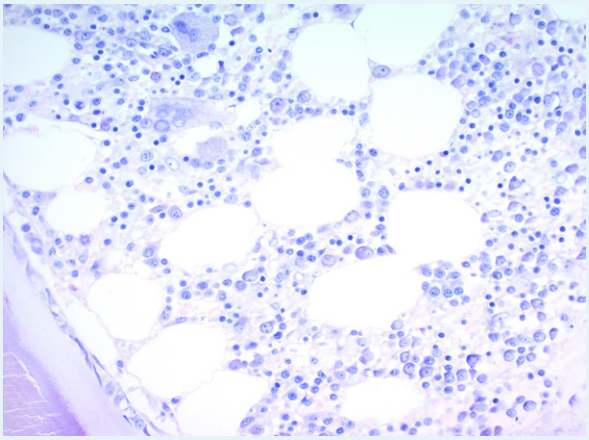
Figure 1. *Bone marrow biopsy, myeloperoxidase stain 200x enlarged, 677 x 508 mm (96 x 96 dpi)*



dpi = dots per inch

Figure 2. *Bone marrow biopsy, Giemsa stain 200x enlarged, 677 x 508 mm (96 x 96 dpi)*



dpi = dots per inch

CASE REPORT

An otherwise healthy 36-year-old Caucasian man presented to the emergency department with a two-day history of fever, nausea, and headache. He was taking ibuprofen for four weeks because of lower back pain. On physical examination, there was nuchal rigidity and fever. Laboratory testing revealed an elevated C-reactive protein of 162 mg/ml, haemoglobin of 9.1 mmol/l, thrombocytes of 258 x 10^9/l, lymphocytes of 0.7x 10^9/l and 0.0 x 10^9/l neutrophilic, eosinophilic, and basophilic granulocytes in the peripheral blood. There was no previous granulocytes count known to be conducted. A lumbar puncture was performed and the spinal fluid revealed low glucose and 1 leucocyte/mm^3. Subsequently, a presumptive diagnosis of meningitis was made and treatment with meropenem initiated. The next day, a bone marrow biopsy was done (figures 1 and 2).

WHAT IS YOUR DIAGNOSIS?

See page 213 for the answer to this photo quiz

ANSWER TO PHOTO QUIZ (PAGE 212)

SOMETHING IS MISSING

DIAGNOSIS

These findings, combined with the lack of granulocytes in the peripheral blood, are consistent with pure white cell aplasia (PWCA), a rare haematological disorder.[1] It is considered an auto-immune phenomenon, with inhibition or destruction of the myeloid precursors of granulocytes. It is not clear if this is predominantly a cellular or antibody-mediated mechanism, but there is (indirect) evidence for both.[1,2] In thymoma-associated disease, there is granulocyte-monocyte colony forming unit (GM-CFU) inhibitory activity in most patients, likely due to antibodies.[3] Thymoma is the disease most often associated with PWCA, but is more often seen in combination with pure red cell aplasia. Other diseases that are reported together with PWCA are primary biliary cirrhosis, Parvo B19 infection, and anti-glomerular basement membrane disease.[3-5] PWCA can also occur as an idiosyncratic drug reaction and ibuprofen is one of the drugs known to cause this.[6] Different treatment strategies directed at the cellular and humoral immune system have been proposed. Corticosteroids, intravenous immunoglobulins, cyclosporine, cyclophosphamide, rituximab, plasmapheresis, and granulocytes-colony stimulating factor (G-CSF) have all been used in the management of disease and most were reported to be effective. PWCA sometimes resolves after thymectomy when associated with thymoma.[2,7] However, there is currently no 'evidence-based medicine' strategy to treat PWCA.[3] In this case, further investigation for all known causes of PWCA as listed above yielded negative findings. Human immunodeficiency syndrome was excluded and there were no demonstrable titres of antinuclear antibody and anti-neutrophil cytoplasmic autoantibody. Bone marrow cytogenetic analysis and tests for autoantibodies against G-CSF were not performed. Ibuprofen was attributed as the most likely causative agent since the patient had recently started taking this; there was an absence of other known associated diseases and this has been described before in three published cases. The ibuprofen was discontinued and the patient received human G-CSF (Filgastrim, 48 million units once daily). On day eight of admission, there were granulocytes in his peripheral blood again. One day later, the G-CSF was discontinued and meropenem switched to ceftriaxone and amoxicillin. Spinal fluid and blood bacterial cultures remained sterile. The polymerase chain reaction for enteroviruses, herpes simplex viruses 1 and 2, varicella zoster virus, *Listeria monocytogenes*, *Neisseria meningitides*, and *Streptococcus pneumoniae* in the spinal fluid were negative, as was the cryptococcus antigen test. He was discharged after completing two weeks of antibiotics and strongly advised to never use ibuprofen or other non-steroidal anti-inflammatory drugs again. A re-challenge was deemed too dangerous. After 33 days, during an outpatient follow-up appointment, the patient remained well, did not complain of back pain, and his neutrophilic granulocytes count was 2.6 x 10^9/l.

DISCLOSURES

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

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PHOTO QUIZ

Does it bite?

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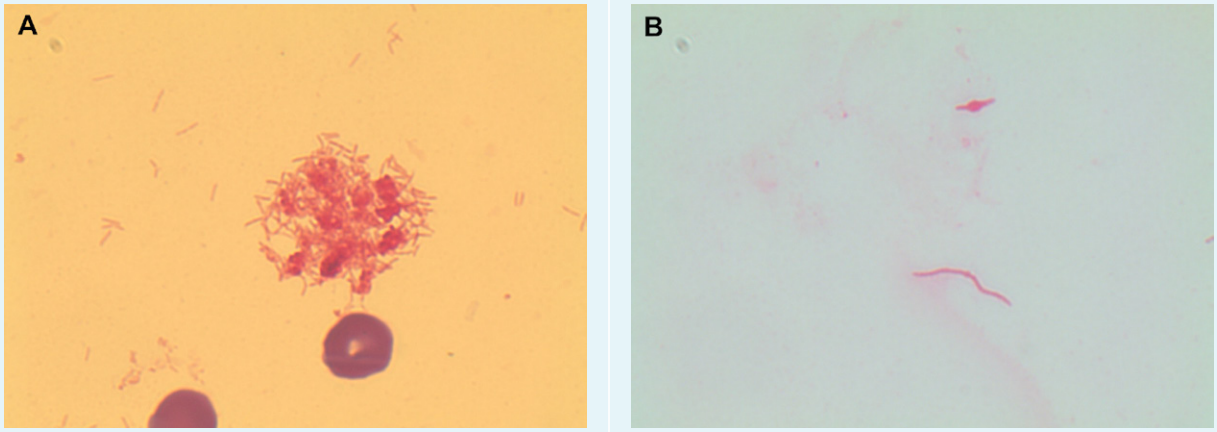
Figure 1. *(panel A) Gram-staining of blood culture, showing multiple Gram-negative rods (1000x magnification); (panel B) Gram-staining of blood culture, showing the long and bended Gram-negative rods more in detail.*



CASE REPORT

A 38-year-old male presented at the Emergency Department with non-bloody diarrhoea and acute abdominal pain for one day. Medical history comprised mild chronic obstructed pulmonary disease and daily use of cannabis and amphetamines.

Physical examination showed a severely ill, disorientated, and euphoric patient. He had a respiratory rate of 32 breaths/minute, an oxygen saturation of 98% with 3 litres of supplemental oxygen, a heart rate of 125 beats/minute, a blood pressure of 138/98 mmHg and a temperature of 36.6 °C. There was no nuchal rigidity. We observed blue coloured lips, diffuse petechiae, purpura, and ecchymosis of the extremities, as well as a small healing wound on the right thumb.

Laboratory investigation showed a haemoglobin concentration of 9.8 mmol/l, leucocyte count of 26.0 x 10^9/l, severe thrombocytopenia with a platelet count of 7 x 10^9/l, D-dimer 21 mg/l, C-reactive protein 452 mg/l, lactate 8.4 mmol/l, creatinine 248 µmol/l, and urea 13.3 mmol/l. Chest X-ray and computed topography scan of the abdomen did not show abnormalities.

He was admitted to the Intensive Care Unit (ICU) with suspected circulatory shock and supported with fluid administration, norepinephrine, and nitroglycerin. During his ICU stay, the haemoglobin concentration decreased to 3.6 mmol/l due to haemolysis (fragmentocytes present in small numbers, direct Coombs test negative, complement C3 and C4 normal). Differential diagnostic considerations were septic shock with diffuse intravasal coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), (Shiga toxin-mediated) haemolytic uremic sydrome (HUS), and (drug-induced) thrombotic microangiopathy (TMA). We commenced treatment with ceftriaxone, ciprofloxacin, gentamicin, and plasmapheresis. On the third day, both aerobic blood cultures bottles showed growth of long bended Gram-negative rods (figure 1).

WHAT IS YOUR DIAGNOSIS?

See page 215 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 214)

DOES IT BITE?

DIAGNOSIS

The positive blood cultures indicated that sepsis with DIC was plausible. ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) activity was 26%, making TTP unlikely, and therefore, plasmapheresis was discontinued. Shigella toxin-mediated HUS was considered unlikely since the duration of diarrhoea was relatively short and the Shigella toxin PCR on stool was negative, as were cultures of urine, faeces, and sputum. Drug-induced TMA is very rare, and the anamnesis and a negative urine drugs/toxicology test indicated that a serotonin toxidrome was unlikely. Echocardiography showed no signs of endocarditis.

Our patient improved with fluid resuscitation, vasopressor therapy, and antibiotics. Creatinine temporarily increased to 666 µmol/l, but no kidney replacement therapy was needed, and creatinine normalised over time. After six days of ICU treatment, the patient was discharged to the general ward and recovered completely.

After eight days, the Gram-negative rods were characterised as *Capnocytophaga canimorsus* with matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. The isolate was susceptible to penicillin and cephalosporins. The wound on the right thumb turned out to be the result of a bite from his puppy, prior to presentation.

C. canimorsus is a fastidious, slow-growing capnophilic Gram-negative rod and inhabits oral cavities of dogs, cats, and other animals. It can cause human infections, usually after a dog bite, but also after licks or just close proximity to animals. Septicaemia often has a fulminant course and a mortality rate up to 31%.[1,2] Severe cases of septic shock, diffuse purpura, and DIC, as in our patient, have been previously reported.[3,4] Although severe infections usually occur in immunocompromised patients (e.g., asplenia, alcohol abuse), this was not the case in our patient.

This case and other cases show that diagnosis of *C. canimorsus* septicaemia is challenging due to incomplete history taking regarding contact with animals, and slow growth of this bacterium.[1,3,5]

C. canimorsus isolates are typically susceptible to penicillin, although there is evidence for increasing resistance due to emerging β-lactamase production. First-line treatment in severely ill patients should be with β-lactamase inhibitor combination, cephalosporins, or carbapenems. When susceptibility to penicillin is proven, a switch can be made, and less severe infections can be treated with clindamycin.[6,7]

DISCLOSURES

The authors have no conflicts of interest to declare.

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LETTER

Utility of salivary gland ultrasonography in primary Sjögren syndrome

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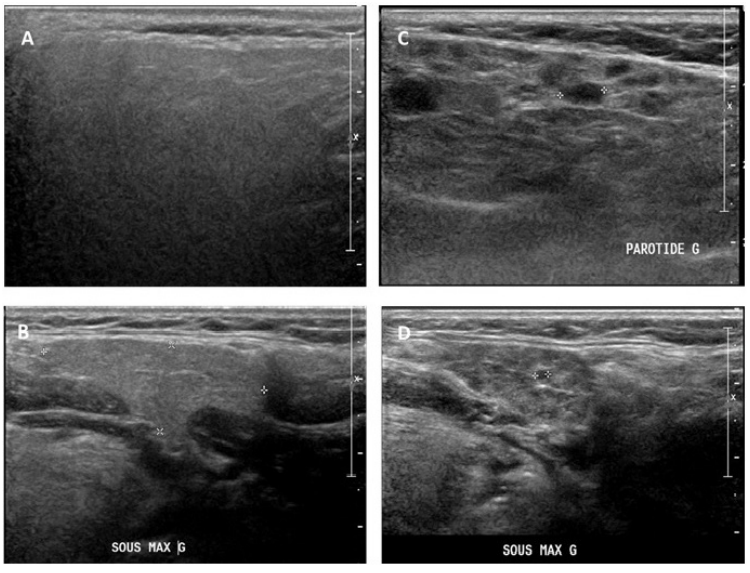
Dear Editor,

Primary Sjögren syndrome (pSS) is a chronic autoimmune disease characterised by sicca syndrome. The diagnosis of SS relies on immunological and histological criteria,[1] and salivary gland ultrasonography (SGUS) appears to contribute to diagnosis and follow up of pSS.[2] The objective of our study was to assess the usefulness of SGUS in diagnosing SS. We performed SGUS for patients with pSS and controls suffering from sicca syndrome not fulfilling American-European Consensus Criteria for pSS.[3] A positive SSA/SSB antibody test or a positive biopsy of minor salivary gland (focus score > 1) was requested to confirm pSS. Written informed consent was obtained from all patients.

The parotid and submandibular glands were examined by ultrasonography. The modified scoring system described by Cornec et al.[4] was used to evaluate echogenicity and homogeneity for each gland. It ranged from grade 0 (normal gland) to grade 4 (multiples hypoechoic area or calcifications). A score > 2 was considered abnormal.

We enrolled 55 patients (30 pSS and 25 controls). In the pSS group, 22 patients had positive minor salivary gland (MSG)

Figure 1. *Ultrasonography scan (US) of major salivary gland. US of the parotid gland (A) and the submandibular gland (B) in a control subject: normal echostructure and homogenous parenchyma. Parenchyma shows irregular contours, multiple hypoechoic areas, and multiple cysts with echogenic bands in the parotid gland (C) and submandibular gland (D).*



biopsy and 25 patients had positive SSA antibodies. Causes of sicca syndrome in the sicca group were diabetes (n = 5), idiopathic origin (n = 10), drug-induced (n = 4), sarcoidosis (n = 4), and hypothyroidism (n = 2). The two groups were comparable in terms of demographic characteristics. Twenty-one patients with pSS had an ultrasonography scan (US) score ≥ 2 and only three patients in the sicca group had a US score ≥ 2 (p = 0.001) (figure 1). The optimal cut-off for a US score was set at ≥ 2, with a sensitivity of 73% and specificity of 88%.The overall US score was correlated directly with SSA antibodies (r = 0.4, p = 0.002), MSG biopsy (r = 0.3, p = 0.002), and the diagnosis of pSS (r = 0.43, p = 0.001). Our study confirms the value of SGUS in contributing to a diagnosis of pSS. Our results suggest that SGUS could avoid MGS biopsy in 21 patients with pSS.

A group of experts suggested that SGUS had a similar weight to minor criteria of 2016 American College of Rheumatology/European League Against Rheumatism classification and improved sensitivity in diagnosing pSS.[5] Other reports demonstrated that abnormal SGUS is associated with high disease activity and damage in pSS, suggesting that SGUS may be integrated in prognostic and therapeutic algorithms.[6]

Our study, although limited by the small number of cases, puts forward the usefulness of SGUS in diagnosing SS by detecting functional and structural impairments. SGUS abnormalities, in combination with clinical and immunological arguments, seems to be helpful for the diagnosis of SS.

DISCLOSURE

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

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LETTER

COVID-19 and vitamin D deficiency, a fatal combination?

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Dear Editor,

On February 27th, 2020, the novel coronavirus (COVID-19) infection was diagnosed in a Dutch patient. Since then, citizens of the Netherlands have been inundated with advice. All intended to slow down the spread of the disease and perhaps make it less fatal.

On March 15th, 2020, the National Institute for Public Health and the Environment (RIVM) reported that 20 people had died from the virus. Most were elderly, older than 70 years of age, and they had underlying health conditions such as diabetes, cardiovascular or pulmonary disease. My question is: "How was the vitamin D status of these people?" This vitamin, in addition to bone and calcium homeostasis, modulates the immune response and thereby reduces the susceptibility to acute respiratory tract infection.[1]

According to the Health Council of the Netherlands, elderly people are vitamin D deficient. They require a daily vitamin D supplement of 10 to 20 micrograms in order to reduce the risk of bone fractures. Furthermore the Council defines subgroups for people between the ages of 4 and 70 and advises them to take an additional 10 micrograms of vitamin D daily. These subgroups are: children, adolescents, and adults who have hardly any daily exposure to the sun, or who avoid exposure to sunlight or wear concealing clothes.[2] Why has this advice not been repeated during this epidemic? It would feed two birds with one scone.

However, it is even not too late for the vitamin D deficient patient in the ICU. Wang et al. describe that COVID-19 patients treated in the ICU, compared with patients not treated in the ICU, have significantly higher serum procalcitonin levels.[3] Fortunately, three years earlier, an Iranian randomised double-blind, placebo-controlled trial demonstrated that intramuscular vitamin D significantly lowers the mortality rate in vitamin D deficient patients with ventilator-associated pneumonia and high serum procalcitonin levels.[4]

Summarising, elderly people should be strongly advised to take a daily vitamin D supplement of 10 to 20 micrograms during this epidemic. In COVID-19 patients, the vitamin D status should be evaluated and in case of a deficiency, parenteral vitamin D given.

DISCLOSURE

The author declares no conflicts of interest. No funding or financial support was received. The author declares to have a few shares in a company producing vitamin D.

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