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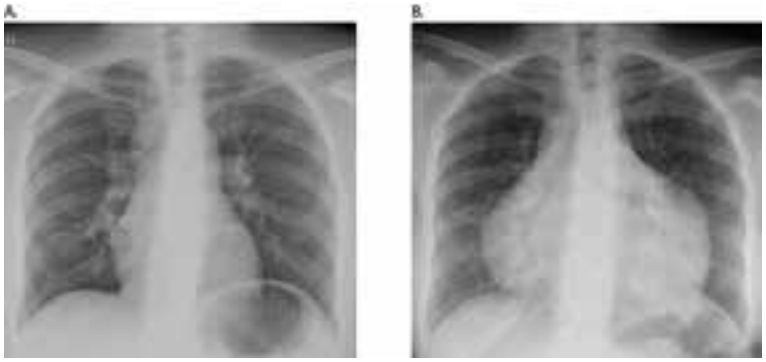

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EDITORIAL

The true impact of a scientific paper

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Scientific work is being published to share the findings from research with a broad audience of colleague scientists, who can build on the new insights provided by the paper or use the results to formulate new hypotheses. Likewise, scientific findings can be used by practising physicians who are seeking to improve the management of their patients. Results from papers reporting groundbreaking new diagnostic strategies or therapeutic options may be readily adopted and may therefore have a very large impact on day-to-day medicine. Unfortunately, there is no 'impact factor' to measure the importance of such manuscripts. Instead, if we speak about the impact factor in relation to articles and journals, we refer to the number of citations that a paper has received in the first two years after its publication.[1] This may indeed reflect scientific impact as many readers and writers may be impressed by the results but similarly it could reflect ample opposition against the findings that may be thought to be flawed. Both situations will result in an identical 'impact', thus making it a weak parameter of esteem for an article.[2,3] Nevertheless, citation analyses, impact factors, and Hirsh factors (a score that is a composite of the number of papers an author has produced and the number of citations these papers have received) are with us to stay.[4] These parameters seem to fulfil our need to quantify not only the number of papers published but also the 'quality' of these articles and as long as one understands the shortcomings of these scoring tools, there is nothing wrong with that.

Keeping all this in mind, I am happy to report that the estimated impact factor of the Netherlands Journal of Medicine in 2012 has further increased. A preliminary analysis of citations in 2012 results in an impact factor of almost 2.5, a further increase compared with previous years (*figure 1*). This is relevant as this firmly positions the Netherlands Journal of Medicine in a group of periodicals that distinguish themselves from a very large number of journals with an impact factor lower than 2 (*figure 2*). An increasing impact factor leads to an increased number of submissions and therefore potentially more choice for the editors and a higher quality of the accepted papers (with eventually higher impact factors in coming years).

Figure 1. *Impact factor of the Netherlands Journal of Medicine in the last 15 years. In 2011 the impact factor rose above 2.0, which is often considered an important boundary that distinguishes better cited journals from the other journals*

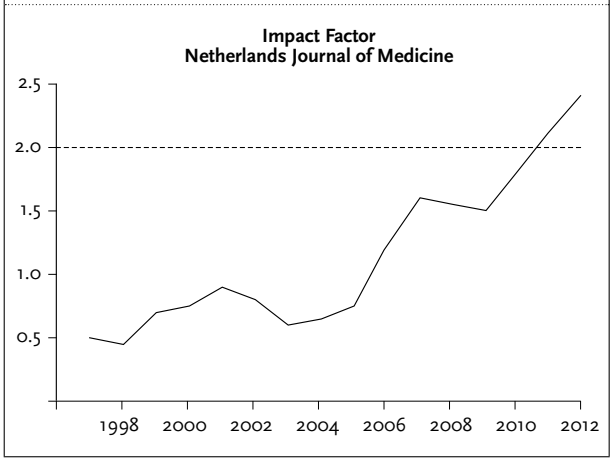
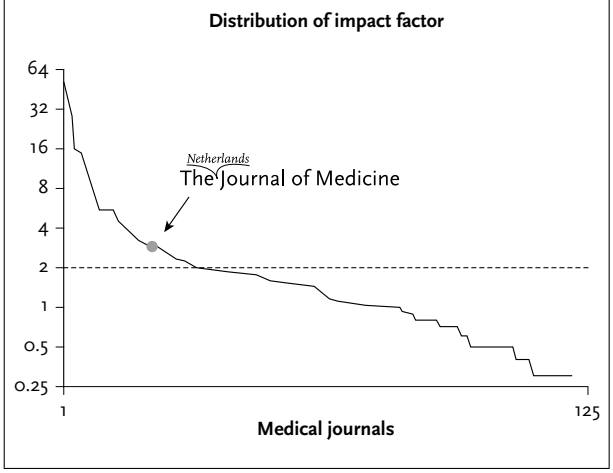


Figure 2. *Position of the Netherlands Journal of Medicine in the group of 125 general medical journals with the highest impact factors. The highest impact factors belong to the New England Journal of Medicine, the Lancet, and JAMA, respectively*



Indeed, the Netherlands Journal of Medicine has seen a larger number of submissions, in particular from other European countries and the US (*table 1*). However, since the number of pages of the journal is more or less fixed, more submissions will result in a lower acceptance rate of submitted papers.[4]. Traditionally, we are publishing the list of articles that have received the largest number of citations in recent years and thereby have contributed most to the impact factor of the Netherlands Journal of Medicine (*table 2*).

Table 1. *Submissions and acceptance rate of the Netherlands Journal of Medicine between 2008 and 2012*

	2012	2011	2010	2009	2008
Submissions	1012	890	555	328	245
Overall acceptance rate	11%	14%	23%	30%	42%
Origin of submissions					
Netherlands	32%	40%	47%	61%	70%
Other European	30%	25%	21%	16%	14%
North America	20%	14%	10%	7%	4%
Rest of the World	18%	21%	22%	16%	12%

Table 2. *Recent papers that contributed most to the impact factor of the Netherlands Journal of Medicine*

de Wijkerslooth T, et al. Strategies in screening for colon carcinoma[5]
Kuiper P, et al. An overview of the current diagnosis and recent developments in neuroendocrine tumours of the gastroenteropancreatic tract[6]
Levi M, et al. Periprocedural reversal and bridging of anticoagulant treatment[7]
Tromp M, et al. The effects of implementation of the Surviving Sepsis Campaign in the Netherlands[8]
Ubbink DT, et al. Implementation of evidence-based practice: outside the box, throughout the hospital[9]
Arends JE, et al. Treatment of acute hepatitis C virus infection in HIV+ patients: Dutch recommendations for management[10]
Gevers TJG, et al. Treatment extension benefits HCV genotype I patients without rapid virological response: a systematic review[11]
Pijl H. Obesity: evolution of a symptom of affluence. How food has shaped our existence[12]
Wouters MM, et al. Neuroimmune mechanisms in functional bowel disorders[13]
Szekanecz Z, et al. Chemokine and chemokine receptor blockade in arthritis: a prototype of immune-mediated inflammatory diseases[14]

We are pleased with the increasing impact of the Netherlands Journal of Medicine and with the increasing number of submissions the journal receives. It may be an illustration of the vitality of the journal and the type of general articles it publishes. The editorial board will do everything in its power to keep the journal in good shape in the years to come.

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REVIEW

Familial hypercholesterolaemia: new treatment options

M. Ezzahti*, E.J.G. Sijbrands, M.T. Mulder, J.E. Roeters van Lennep

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ABSTRACT

Familial hypercholesterolaemia is a relatively frequently occurring disease that is strongly associated with vascular disease. Current treatment with cholesterol-lowering agents is partly effective but shows variable responses between patients with familial hypercholesterolaemia. Recently, new cholesterol-lowering drugs have been developed. Here we describe the most promising of these new agents for which results from phase 2 or phase 3 trials are available. We will discuss the data regarding lipid-lowering potential and safety issues and speculate about the potential reductions of the residual risk of statin-treated FH patients.

KEYWORDS

Familial hypercholesterolemia, treatment, PCSK9, apolipoprotein B, synthesis inhibitor, CETP, MTP

INTRODUCTION

Familial hypercholesterolaemia (FH) is an autosomal dominant disorder. More than 85% of FH cases are due to mutations in the low-density lipoprotein-receptor (LDL-R) encoding gene.[1] More than 1000 different mutations in the LDL-R encoding gene have been described. The LDL-R is responsible for clearing LDL cholesterol (LDL-C) from the blood by endocytosis and intracellular degradation. If the receptor is defective or lacking, this will lead to reduced clearance of cholesterol. As a result the endogenous cholesterol synthesis in the liver is increased, enhancing very low-density lipoprotein (VLDL) production. FH is a co-dominant disease. Heterozygous FH is relatively common in Caucasian populations (1:450-500) whereas homozygous FH is rare (1:1.000,000).[2] Occasionally, FH may also be caused by mutations in the genes encoding for apolipoprotein B-100 (Apo B-100) and the proprotein convertase subtilisin/kexin type 9 (PCSK9).[1,3] The diagnosis of FH is based on clinical criteria or by detection of a pathogenic mutation in the *LDL-R, Apo B* or *PCSK9* gene.[4] FH patients are at increased risk for premature cardiovascular disease (CVD): clinical manifestations of CVD occur in 50% of untreated male heterozygous FH patients before the age of 50 years and in women before the age of 60 years.[5]

According to the current guidelines, the treatment goal for FH patients is lowering LDL-C <2.5 mmol/l for primary cardiovascular prevention and <1.8 mmol/l in the secondary prevention setting.[6] The mainstay of treatment is lifestyle modifications and statin therapy. And classical cardiovascular risk factors should be treated aggressively in FH patients.

Statins inhibit hepatic cholesterol synthesis and indirectly raise expression of LDL-Rs.[7] Statins effectively reduce CVD mortality and morbidity in FH patients.[8-12] In primary prevention, statin therapy in FH patients reduced total coronary heart disease (CHD) by 48%.[13] The reduction of CHD mortality by statins in patients with a history of symptomatic CHD was much smaller, namely 25%.[13] Only 21% of patients with FH in the Netherlands achieved the treatment goal of LDL-C <2.5 mmol/l.[14] This treatment goal remains difficult to reach despite the use of high-dose statins, even in combination with other cholesterol-lowering drugs. The LDL-lowering capacity of statins in combination with other lipid-lowering drugs is maximally around 50-60%.[15] FH patients have such a strongly elevated LDL-C that in most cases maximal current treatment is not sufficient to reach the mentioned LDL targets. Moreover, side effects, especially myalgia without CK elevation, occur frequently: in 5-10% of all statin-treated patients.[15] This is a growing problem in routine clinical practice. Therefore,

FH patients still have a large residual CVD risk despite the use of statins and there is a medical need for new additional drugs to further lower LCL-C in patients with FH to improve the prognosis of these patients.

Recently, new cholesterol-lowering drugs have been developed.[16] In the current review we describe the most promising of these new therapies of which results from phase 2 or phase 3 trials are available. We will discuss the data regarding lipid-lowering potential and safety issues and speculate about the potential reductions of the residual risk of statin-treated FH patients.

NEW THERAPEUTIC OPTIONS

PSCK9 targeted therapy

Proprotein convertase subtilisin/kexin type 9 (PSCK9), a serine protease, stimulates the lysosomal degradation of the LDL-R within hepatocytes.[17] PCSK9 is synthesised primarily in the liver. PCSK9 binding to the LDL-R causes degradation of the LDLR-LDL complex in the lysosomes which leads to a decreased number of available LDL-Rs (*figure 1*).[18] Therefore, gain-of-function mutations in the *PCSK9* gene cause hypercholesterolaemia.[19] And loss-of-function mutations result in low levels of LDL-C and lead to an 80% CVD risk reduction.[20-22] These findings made PCSK9 extremely interesting as a new target for lipid-lowering therapy. Different therapeutic strategies to lower PCSK9 concentration are in development, such as monoclonal antibodies to PCSK9, small interfering RNAs and antisense oligonucleotide-based therapy. Most human studies have been performed with the monoclonal antibody variants so far.

REGN727 is a fully human monoclonal antibody highly specific for human PCSK9 which blocks its interaction with the LDL-R. Recently, Stein *et al.* reported a multicentre, randomised, placebo-controlled phase 2 trial conducted in 77 high-risk patients with heterozygous FH.[23] In this dose-finding study, the cholesterol-lowering effect and safety of REGN727 was evaluated. All patients were treated with high doses of statins and 71% of the patients were also using ezetimibe. The mean reductions in LDL-C after 12 weeks of subcutaneous REGN727 ranged dose-dependently from 29% to 68% compared with 11% in the placebo group. The most effective dose was 150 mg subcutaneously every two weeks. With this dose 94% of patients reached an LDL-C ≤2.5 mmol/l and 81% an LDL-C ≤1.8 mmol/l. The most common adverse events were mild reactions at injection sites but serious adverse events were not reported. Another study in patients with primary hypercholesterolaemia who had an LDL-C ≥2.5 mmol/l showed that the reduction of LDL-C from baseline was significantly larger in patients receiving either atorvastatin 10 mg + REGN727 150 mg subcutaneously every two weeks or atorvastatin 80 mg + REGN727 150 mg subcutaneously every two weeks compared with atorvastatin 80 mg + placebo (66.2% and 73.2% *vs* 17.3%, p<0.001).[24] Of the patients receiving REGN727, 100% and more than 90% of patients attained an LDL-C of ≤2.5 mmol/l and ≤1.8 mmol/l, respectively, compared with 52% and 17% of those patients receiving atorvastatin 80 mg alone.

REGN727 is the first of the PSCK9 inhibitors to show significant LDL-C reduction, is well tolerated and showed a favourable safety profile during short-term administration in FH patients (*table 1*).

AMG145, another monoclonocal antibody to PCSK9, was recently evaluated in a phase 2 study and showed promising results in statin-intolerant patients. The mean reductions in LDL-C after 12 weeks of subcutaneous AMG145 ranged dose-dependently from 41% to 63% compared with 15% in the ezetimibe/placebo group.[25] The RUTHERFORD study evaluated AMG145 in patients with heterozygous FH with statin therapy with or without ezetimibe. After 12 weeks of therapy, the mean LDL-C reduction was 43% and 53% with AMG 145 350 mg and 420 mg respectively, compared with 3% increase with placebo.[26] And in this trial, 70% and 89% of the FH patients reached the target LDL levels with 350 mg and 420 mg respectively. AMG145 was administered subcutaneously every four weeks in both studies, with minimal adverse events. In the coming years, studies addressing the effect of different strategies for injections (frequency, dose) will be performed.

Future studies on mortality and morbidity are required to determine the role of this promising approach in the treatment of FH. If these studies show that this new treatment option is indeed as effective and safe as the results indicate so far, then this class of medication will have a major impact on the number of FH patients who can reach

Figure 1.

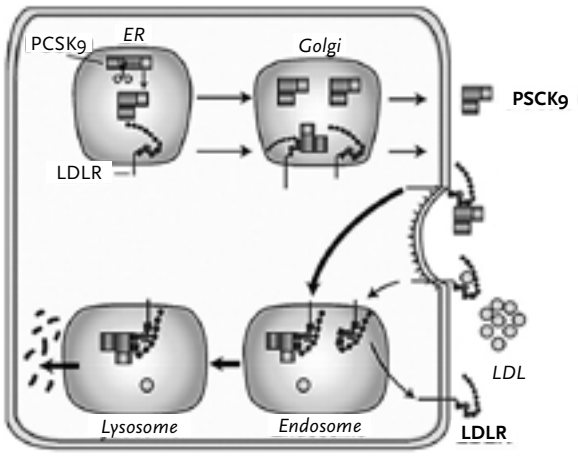


Table 1.

Therapy	Study	Patients	Design	Outcome	Results	Adverse events
REGN727	Stein et al.[23]	Hetero-zygous FH n=77	Phase 2 study: randomised, placebo-con-trolled trial	LDL-C reduction after 12 weeks	**Dose 150 mg every 2 weeks:** **LDL-C** Placebo: -10.65% REGN727: -67.9% (p<0.0001) **ApoB100:** Placebo: -6.39% REGN727: -50.19% (p<0.0001) **Lp(a):** Placebo: -3.91% REGN727: -23.38% (p=0.2559)	**Dose 150 mg every 2 weeks:** **Injection site reactions:** Placebo: 13% REGN727: 44%
REGN727	Roth et al.[24]	Primary hyper-cholesterol-aemia n=92	Phase 2 study: randomised, placebo-con-trolled trial	LDL-C reduction after 8 weeks	**Atorvastatin 80 mg + placebo:** -17.3% **Atorvastatin 10 mg + REGN727:** -66.2% **Atorvastatin 80 mg + REGN727:** -73.2%	**Injection site reactions**: Atorvastatin 80 mg + placebo: -6.5% Atorvastatin 10 mg + REGN727: -0% Atorvastatin 80 mg + REGN727: -3.3%
AMG145	Raal et al.[26]	Hetero-zygous FH n=77	Phase 2 study: randomised, placebo-con-trolled trial	LDL-C reduction after 12 weeks	**Placebo:** +3% **AMG145 350mg:** -43% **AMG145 420 mg:** -55%	**No difference between groups**

their treatment goals. The hope is that this will lead to a reduction in residual risk of CVD, especially in secondary prevention. Whether PCSK9 targeted therapy leads to LDL-C reduction in homozygous FH patients with no LDL-receptor function is doubtful because of its mode of action.

Inhibitors of apolipoprotein B synthesis

Apo B-100 is an essential component of LDL-C and all other atherogenic lipoproteins. Apo B-100 is the main ligand for the LDL-R.[27] Inhibition of apo B production is therefore an interesting concept to lower the production of atherogenic lipoproteins with the objective of reducing risk of CVD in FH patients.

Mipomersen is the first apo B synthesis inhibitor for human use. Mipomersen is a second-generation antisense oligonucleotide and inhibits the synthesis of apo B-100.[28] This antisense oligonucleotide binds to the mRNA of apo B-100, thereby stimulating the degradation of apo B by endogenous RNase H. This results in reduced synthesis of apo B and a decrease in very-low-density lipoprotein (VLDL) and LDL levels. As the mode of action of mipomersen is independent of the presence of LDL-Rs, this therapy is also suitable for homozygous FH patients.

Five randomised double-blind, placebo-controlled trials using mipomersen have been conducted.[29-33] These studies evaluated mipomersen 200 mg once weekly (subcutaneously) in patients with heterozygous FH,[30,31] homozygous FH[29] and severe hypercholesterolaemia with a high risk of CVD.[32,33] The duration of all these studies was 26 weeks and the primary outcome was LDL-C reduction. Mipomersen was administered in combination with other lipid-lowering drugs, including statins. Mipomersen 200 mg/week resulted in a significant reduction in LDL-C between 21% and 47% compared with placebo.[29-33] Mean apo B and lipoprotein (a) were also reduced by 20% to 35%. The reduction in LDL-C, apo B and lipoprotein (a) seemed to be independent of the underlying type of hypercholesterolaemia and independent of concomitant drug therapy.

Mipomersen was reasonably well tolerated in the five studies, despite the fact that the majority of the patients had mild to moderate injection site reactions (*table 2*). This side effect is dose-dependent, occurs within 24 hours of the drug injection and is in general transient.

Because of the mode of action of mipomersen, accumulation of hepatic fat is a serious concern. Elevated

Table 2.

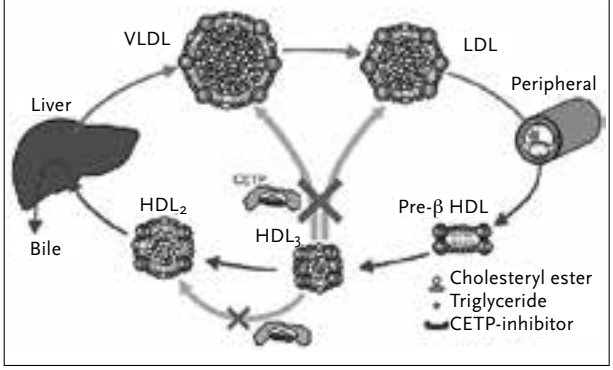
Therapy	Study	Patients	Design	Outcome	Results	Adverse events
Mipomersen	Raal et al.[29]	Homozygous FH n=51	Phase 3 study: randomised, placebo-controlled: mipomersen 200 mg/week for 26 weeks	LDL-C reduction, reduction of apo B, HDL-C, triglycerides and lipoprotein (a)	**LDL-C:** Placebo: -3.3% Mipomersen: -24.7% (p=0,0003) **ApoB100:** Placebo: -2.5% Mipomersen: -26.8% (p=0.0001) **Lp(a):** Placebo: -7,9% Mipomersen: -31.1% (p=0.0013)	**Injection site reactions:** Placebo: 24% Mipomersen: 70% **Flu-like symptoms:** Placebo: 24% Mipomersen: 29% **Elevated transaminases** (>3 ULN): Placebo: 0% Mipomersen: 12%
Mipomersen	Stein et al.[30]	Heterozygous FH n=124	Phase 3 study: randomised, placebo-controlled: mipomersen 200 mg/week for 26 weeks	LDL-C reduction, reduction of apo B, HDL-C, triglycerides and lipoprotein (a)	**LDL-C:** Placebo: -5.2% Mipomersen: -34% (p=0.001) **ApoB100:** Placebo: +7% Mipomersen: -26.3 (p=0.001) **Lp(a):** Placebo:+2.4% Mipomersen: -20.4% (p=0.00)	**Injection site reactions:** Placebo: 42% Mipomersen: 93% **Flu-like symptoms:** Placebo: 39% Mipomersen: 42% **Elevated transaminases** (>3 ULN): Placebo: 0% Mipomersen:6%
Mipomersen	McGowan et al.[31]	Severe heterozygous FH and high risk for atherosclerosis n=58	Phase 3 study: randomised, placebo-controlled: mipomersen 200 mg/week for 26 weeks	LDL-C reduction, reduction of apo B, HDL-C, triglycerides and lipoprotein (a)	**LDL-C:** Placebo: +6% Mipomersen: -36% (p=<0.001)	**Injection site reactions:** Placebo: 32% Mipomersen: 90% **Flu-like symptoms:** Placebo: 21% Mipomersen:46% **Elevated transaminases** (>3 ULN): Placebo: 0% Mipomersen:15%

alanine aminotransferase (ALAT >3 x upper limit of normal) occurred in 6% to 15% of patients treated with mipomersen.[29-33] The elevated liver transaminases were not accompanied by other findings of hepatic dysfunction. Elevated ALAT most likely reflects accumulation of hepatic fat secondary to inhibition of apo B and therefore VLDL. Hepatic steatosis has indeed been observed in a number of mipomersen-treated patients.[34] After discontinuation of treatment with mipomersen, the elevated ALAT returned to normal and hepatic steatosis resolved in all patients within ten weeks.[34]

Recently the Federal Drug Agency (FDA) approved mipomersen for the treatment of homozygous FH patients. However, the European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) gave a negative opinion on approving mipomersen because of safety concerns, specifically accumulation of hepatic fat. Currently, a phase 3 trial in severe heterozygous FH patients and an early access program for homozygous FH patients are still going on in Europe.

In summary, mipomersen seems an effective additional therapy on top of statins in patients with severe hypercholesterolaemia. However, because of the side effects and the lack of morbidity and mortality data, it is unlikely that mipomersen will be available in Europe within the near future.

Figure 2.



Cholesterol ester transfer protein inhibitors

Cholesterol ester transfer protein (CETP) is a plasma glycoprotein that is bound to HDL particles and promotes the exchange of cholesteryl esters between HDL and triglyceride-rich lipoproteins.[35-37] Inhibition of CETP raises HDL-C and decreases LDL-C (*figure 2*), leading to a more favourable lipid profile which could reduce the risk of CVD in FH patients.[38] Torcetrapib, the first CETP inhibitor, was tested in FH patients. However, all torcetrapib trials were terminated because torcetrapib was associated with an excess mortality in patients at high risk for CVD in the large Illuminate study.[38,39] Torcetrapib was also associated with increased blood pressure and aldosterone levels.[40] These adverse effects were unrelated to the inhibition of CETP and were considered to be molecule-specific.[41,42] New CETP inhibitors, such as dalcetrapib and anacetrapib, did not show an effect on blood pressure. After promising safety data,[43,44] the dal-OUTCOMES trial was unexpectedly stopped in May 2012 after an interim analysis showed that dalcetrapib was not significantly reducing CVD events in patients with hypercholesterolaemia.[45,46] Recently, the DEFINE study showed in a secondary prevention setting that in addition to statin therapy, anacetrapib 100 mg daily compared with placebo significantly decreased LDL-C by 36% and significantly increased HDL-C by 138% after 24 weeks.[47] The REVEAL study is currently testing whether anacetrapib reduces the number of CVD events in atorvastatin-treated patients with a history of CVD.[48] The new CETP inhibitors were not specifically tested in FH patients. However, if anacetrapib is effective and safe in patients with hypercholesterolaemia, anacetrapib is also likely to benefit FH patients.

Microsomal triglyceride transfer protein inhibitors

Microsomal triglyceride transfer protein (MTP) is primarily an enzyme responsible for transfer of triglycerides from their site of synthesis into the lumen during the assembly of VLDL and chylomicrons. VLDL is the major source of LDL in plasma.[49] Blocking MTP reduces LDL-C, VLDL and triglycerides by affecting the packaging and secretion of VLDL and chylomicrons.[50] Because the mechanism of action does not involve LDL-Rs, this treatment is also suitable for homozygous FH patients.

Phase 2 studies showed that lomitapide treatment in homozygous FH patients results in a reduction in LDL-C of 50% to 60%. In these patients liver-fat content increased during the use of lomitapide, which at the highest dose ranged from less than 10% to more than 40%.[51,52] A phase 3 study was conducted by Cuchel *et al*. in 29 patients with homozygous FH to assess LDL-C reduction and safety of lomitapide.[53] These patients used different combinations of lipid-lowering therapies, including LDL apheresis in 62% of the patients. The median dose of lomitapide in this study was 40 mg. LDL-C was reduced by 50% after 26 weeks of therapy with lomitapide. This effect was sustained and remained stable after 78 weeks of therapy. Similar reductions were observed for apo B. Gastrointestinal symptoms were the most common adverse event and occurred in 93% of patients, but the symptoms were usually mild. About 14% of patients had elevations in liver aminotransferases, but they could all continue lomitapide after dose reduction or temporary suspension of the therapy. Hepatic fat content increased by approximately 7% after 78 weeks of therapy. Since the clinical significance and long-term implications of the increase in hepatic fat as a result of lomitapide therapy is not clearly understood, rigorous and standardised long-term monitoring will be necessary.

In analogy of mipomersen, lomitapide is currently approved by the FDA for the treatment of homozygous FH patients. The approval procedure by the EMA CHMP is currently ongoing. Lomitapide is a promising drug for patients with homozygous FH: the benefit-risk ratio of lomitapide in patients with homozygous FH, who are at a high risk of cardiovascular events and death at a young age, could possibly be favourable. Future studies are needed to evaluate the long-term safety of lomitapide.

Other therapies in development

A novel lipid-lowering therapy in the development is CER-001, which is a recombinant HDL mimetic and is based on human apolipoprotein A-I, the major structural protein of HDL.[54] CER-001 is designed to mimic HDL, which removes cholesterol from tissues and blood vessels and carries it to the liver. Preclinical and clinical data showed efficacy of CER-001 in mobilising cholesterol and promoting reverse lipid transport.[55] The available drugs to treat FH are targeted at reducing LDL-C. These measures can retard the progression of cardiovascular disease; however, they are unlikely to lead to regression of existing disease due to years of cholesterol accumulation in the vessel walls. HDL has multiple actions that could lead to plaque stabilisation and regression. Currently, the effect on plaques is being evaluated in a study in which CER-001 infusion is being tested in homozygous FH patients. Even if this new therapy is shown to be efficacious and safe, the route of administration will certainly limit widespread use.

Recently, significant progress in gene transfer technology has encouraged investigators to further develop LDL-R gene transfer approaches for the treatment of FH. The advantage of gene therapy over other therapeutic regimes is the potential for lifetime correction with a single vector administration. In experimental animal models of FH, LDL-R overexpression following viral vector-based gene transfer resulted in long-term stable correction

of hyperlipidaemia, with attenuation of atherosclerosis progression, and in certain cases even with lesion regression.[5,6] Despite the considerable progress, no viral vector so far is ideal for *in vivo* gene transfer. Future research will focus on making gene transfer vectors safer and more efficient.

CONCLUSIONS

There are many new lipid-lowering drugs for FH patients in development. The drugs we have described in this review are currently the furthest developed, although data on cardiovascular endpoints are not available for most of these agents. These new drugs have an impressive lipid-lowering ability. So far, PCSK9 inhibitors have shown very few side effects, which makes them attractive for a large group of FH patients. Other drugs will be probably reserved for the most severely affected FH patients, such as homozygous FH patients, because of the severity of the side effects and the expectation that PCSK9 inhibitors will have no effect in patients without LDL-R function. With the development of new drugs, the possibilities of treating FH patients to target will improve enormously. This will bring us closer to the ultimate goal: to abolish the residual risk of FH patients, thereby reducing the risk of CVD in FH patients.

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REVIEW

Haemodynamic monitoring of morbidly obese intensive care unit patients

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ABSTRACT

Because of technical and practical difficulties in relation to increased body size, haemodynamic monitoring of morbidly obese critically ill patients (i.e. body mass index ≥40 kg/m²) may be challenging. Obese and non-obese patients are not so different with respect to haemodynamic monitoring and goals. The critical care physician, however, should be aware of the basic principles of the monitoring tools used. The theoretical assumptions and calculations of these tools could be invalid because of the high body weight and fat distribution. Although the method of assessing haemodynamic data may be more complex in morbidly obese patients, its interpretation should not be different from that in non-obese patients. Indeed, when indexed for body surface area or (predicted) lean body mass, reliable haemodynamic data are comparable between obese and non-obese individuals.

KEYWORDS

Haemodynamic monitoring, critically ill patients, intensive care unit, obesity, cardiac output, vascular access, cardiovascular

INTRODUCTION

Obesity, in its epidemic presentation, is still increasing in the industrialised world.[1] The prevalence of obesity in intensive care units (ICUs) has increased over recent years as well. Its prevalence has been estimated from 5 up to 25% and is associated with substantial morbidity and mortality.[1-4]

The degree of obesity is traditionally assessed by body mass index (BMI), which is the ratio of weight (in kilograms) to height (in meters squared). Current definitions and terms of obesity are: overweight: BMI 25.0-29.9 kg/m²; class I obesity: BMI 30.0-34.9 kg/m²; class II obesity: BMI 35.0-39.9 kg/m²; and class III obesity: BMI ≥40 kg/m². Class III is referred to as severe, extreme or morbid obesity.[5] Reasons to initiate any form of haemodynamic monitoring in critically ill patients are multiple.[6] The identification of a disease state and/or its complications is one major reason. Another reason is to understand the aetiology of the disease, usually shock-like pathology. Pending the cause of shock (hypovolaemic shock, cardiogenic shock, obstructive shock or distributive shock), treatment will be different and should be individualised. In line with this, another reason is to tailor treatment, according to the underlying disease, to improve supply, and meet the metabolic demands of the tissues. The ultimate goal is to improve the patient's outcome. Notably, there is a tendency in time, mostly for safety reasons, to develop and use more non- and/or semi-invasive devices for continuous haemodynamic monitoring.

Indications for haemodynamic monitoring in critically ill patients are probably quite similar between obese and non-obese patients. The critical care physician, however, may be confronted with unique and challenging problems in morbidly obese patients related to changes of anatomy and fat distribution with higher body weights.[7] Literature on haemodynamic monitoring in morbidly obese patients is scarce and sketchy. Haemodynamic monitoring protocols for the morbidly obese population differ between institutions and are frequently based on individual experiences. Moreover, most protocols are based on information from studies and reports derived

from perioperative care in bariatric surgery. It has to be considered that the reasons and goals with respect to haemodynamic monitoring in perioperative care and care in ICUs are different. Perioperative care focuses on support for a short time to overcome surgery. On the other hand, care in ICUs is more closely focussed toward diagnostic and therapeutic goals. It is, for that reason, questionable to what extent results from bariatric surgery patients can directly be extrapolated to critically ill obese patients.
As described earlier by others[8] and by our group,[9] in this manuscript we will discuss techniques for haemodynamic monitoring, as well as means to gain vascular access, in the critically ill obese patient.

CARDIOVASCULAR ALTERATIONS IN MORBID OBESITY

Cardiovascular alterations in relation to obesity comprise an increase in total blood volume, cardiac output (CO) and stroke volume (SV), linearly with increasing body weight. The heart rate is unaffected. Left ventricular end-diastolic diameter is increased (i.e., dilatation) as a result of the increased left ventricular stroke volume.[10] In obese individuals the left ventricular systolic function is depressed although left ventricular ejection fraction is increased. Right ventricular function seems to be unchanged.[11] Systemic oxygen delivery is increased as a result of increased CO, serving the metabolic demands of excess fat.
The interpretation of haemodynamic data per se should not be complicated in case of morbid obesity.[12] When indexed for body surface area (i.e., cardiac index (CI); CI = CO/body surface area in m^2) the output is not different between obese and non-obese individuals, regardless the changes in CO. Other haemodynamic parameters (such as extravascular lung water measurement) may be indexed to predicted lean body mass, expressed as $ml.kg^{-1}$. In view of the cardiovascular effects of obesity, however, precautions in data interpretation between obese and non-obese patients must be taken into account, considering the technical and physiological principles and the algorithms of the applied haemodynamic monitoring devices.

STANDARD HAEMODYNAMIC MONITORING

Table 1 summarises standard methods of haemodynamic monitoring.

Physical examination

Central venous pressure (CVP) estimation by physical examination in non-obese patients is known to be difficult, resulting in a high inter- and intra-observer variability. In obese patients CVP measurement is even more difficult as CVP estimation may be obscured due to the high body weight. Jugular venous distension, hepatojugular reflex, cannon waves (as a sign of atrioventricular dissociation), and tricuspid regurgitation may be diagnosed at the bedside. Low body (core) temperature and decreased diuresis in obese patients, similar to that in non-obese patients, may indicate insufficient circulation.

Non-invasive arterial blood pressure measurement

Arterial blood pressure measurement is an essential part of haemodynamic monitoring. Non-invasive blood pressure measurements (oscillometric or auscultatory by sphygmomanometry), however, are shown to be inaccurate in obese patients when compared with intra-arterial blood pressure readings.[13] For accurate blood pressure measurement it is essential to use of a proper-sized blood pressure cuff in obese patients. The length of the bladder should be at least 75 to 80% of the circumference of the upper arm, and the width of the bladder should be more than 50% of the length of the upper arm and approximately 40% of the circumference of the upper arm.[14,15] Systolic pressure may be overestimated if too small a cuff is used.
Blood pressure and heart rate may alternatively be monitored non-invasively from the radial artery (e.g., with the Vasotrac system).[16] The Vasotrac measurements were found to differ considerably compared with intra-arterial measurements in obese patients undergoing bariatric surgery although this method was judged to be more comfortable by patients when compared with oscillometric blood pressure methods.[17]
Alternatively, blood pressures may be measured by ankle monitoring or by Doppler technique when blood pressure readings could not be obtained from the upper extremities. Ankle systolic and mean arterial pressures are significantly higher than brachial blood pressures but severe hypotension may interfere with ankle blood pressure monitoring.[18,19] Systolic blood pressure may be assessed by the Doppler technique as well by use of an appropriate pressure cuff for the legs (calf). After reaching the systolic blood pressure Doppler tones will be audible at the foot arteries (i.e., arteria dorsalis pedis, arteria tibialis anterior) during de-sufflation of the cuff.

Invasive arterial blood pressure measurement (catheterisation)

Intra-arterial measurement of systemic blood pressure (direct measurement) is nowadays a routine procedure in the ICU. Besides blood pressure measurement, arterial cannulation facilitates repeated samplings of arterial blood gas analysis, which permits frequent assessment of oxygenation/gas exchange and ventilation, as well as acid-base monitoring. Arterial cannulation, however, may be difficult in obese patients because of the obscured anatomic landmarks and concomitant peripheral oedema.

Table 1. *Standard haemodynamic monitoring*

Monitoring device	Measurements / parameters	General remarks or problems	Remarks or problems in obese patients
Physical examination: - Central venous pressure	Jugular venous distention Hepatojugular reflex Cannon waves	High intra- and inter-observer variability	Clinical judgement may be obscured by obesity
- Temperature	Tricuspid regurgitation	None	
- Diuresis	Core temperature Urine output	None	
Arterial pressure by sphygmomanometry or oscillometric methods	Systolic and diastolic BP Heart rate Pulsus paradoxus	Reliable, if correct technique used	Reliable, if correct technique used
Arterial catheterisation	Parameters: Systolic BP – Diastolic BP – Mean arterial BP – Heart rate – Pulse pressure – Pulsus paradoxus Arterial blood gas analysis In case of arterial pressure waveform analysis: Stroke volume – Cardiac output – Pulse pressure variation – Stroke volume variation	Standard monitoring tool in ICU	Due to alterations in arterial compliance characteristics, CO by uncalibrated arterial pressure waveform analysis is not reliable but may be used as trend in the obese
Electrocardiography	Heart rate Dysrhythmias Conduction disorders Previous MI Myocardial ischaemia	None	Changes related to obesity (see text)
Pulse oximetry	SpO2 Heart rate	Inaccurate signals by motion artefacts, nail polish, hypotension, low cardiac output, vasoconstriction, hypothermia, haemoglobinopathy	None
Capnography	End-tidal CO2	Related to minute ventilation and cardiac output Check for correct endotracheal ventilation Check for global circulation in case of resuscitation	Higher difference in end-tidal CO2 and arterial CO2 compared with non-obese

In the early 1980s pulse contour analysis was introduced to calculate SV and CO by analysis of the arterial pressure pulse contour (waveform).[20] Pulse contour analysis, as a technique, is based on the assumption that the contour of the arterial pressure waveform is proportional to SV, which can be estimated by the integral of the change in pressure from end-diastole to end-systole over time. This integral calculation comprises several assumptions with respect to individual patient characteristics. These assumptions, for example, implicate demographic variables (such as age, gender, height and weight) and vascular wall characteristics (depicted in skewness and kurtosis of the waveform, summarised in factor X), which may be quite different in obese compared with non-obese patients. It has been shown that uncalibrated pressure pulse contour analysis underestimates SV or CO in obese patients due to systematic errors by these assumptions.[21] For these reasons, uncalibrated CO monitoring should be examined as a trend rather than a single value in obese patients.[22]

Electrocardiography

One of the standard monitoring tools in the ICU is electrocardiography (ECG) or rhythm monitoring. Obesity is associated with a wide variety of ECG abnormalities. After substantial weight loss many of these ECG abnormalities are reversible.[23] ECG alterations with obesity include changes in electrical axes, conduction times (including corrected QT interval) and P, QRS and T wave voltages.

ECG abnormalities may serve as markers of risk for sudden cardiac death but not all ECG alterations are ominous. Some ECG changes, however, represent alterations in cardiac anatomy or morphology associated with obesity and/or its comorbidities. It has to be considered that

obesity, by itself, may invalidate commonly used ECG diagnostic criteria. Criteria for left ventricular hypertrophy, for example, may be invalidated by isolation effects of the body fat, yielding lower QRS-complex voltages.[23]
Obesity has been identified as an arrhythmogenic factor. Obesity is also associated with the occurrence of both supraventricular and ventricular arrhythmias and even sudden cardiac death.[24,25] Atrial fibrillation is the most prevalent supraventricular arrhythmia associated with obesity. Atrial fibrillation in obese patients has been shown to be related to worse clinical outcome.[26,27] Case reports and small studies reported a variety of cardiac arrhythmias and conduction disturbances in obese patients with left ventricular hypertrophy and sleep apnoea syndrome.[28]

Pulse oximetry

Pulse oximetry is, alike ECG, one of the standard monitoring tools in routine critical care medicine.[29] Pulse oximetry is based on spectrophotometric features of pulsatile arterial blood flow and absorption characteristics of oxyhaemoglobin and deoxyhaemoglobin with respect to two different wavelengths of light (660 nm (red light) and 940 nm (infrared), respectively).[30] Pulse oximetry is probably the most valuable non-invasive method to continuously monitor oxygen saturation in patients at risk for hypoxaemia.
There is still debate about the clinical benefit of early detection of hypoxaemia by oximetry in the perioperative period although the use of pulse oximetry in the ICU is considered to be a routine procedure.[31] The pulse oximeter is less reliable in low perfusion states and hypothermia. In case of dyshaemoglobinaemia (e.g. with carbon monoxide poisoning and methaemoglobinaemia) pulse oximeters are not able to measure oxygenation adequately. It has been shown that pulse oximetry does not improve outcome in perioperative care.[31,32] In this respect no differences seem to exist between critically ill non-obese and obese patients.

Capnography

During mechanical ventilation changes in patients' cardiovascular and respiratory status can be assessed by capnography. Reduction in CO results in decreased end-tidal CO_2 ($EtCO_2$) values and in increased end tidal-arterial CO_2 differences. In case of effective cardiopulmonary resuscitation, increases in end-tidal CO_2 signify an increase in CO (i.e. pulmonary capillary blood flow). In mechanically ventilated obese patients, capnography may be helpful to assess (changes in) the patient's global haemodynamic status.[33] In spontaneously breathing, unintubated patients sampling $EtCO_2$ through a nasal cannula is potentially problematic when expired gas mixes with ambient air. The resulting inaccurate measurements produce artificially low values compared with a closed system with minimal dead space. Intermittent mouth breathing might also contribute to underestimated $EtCO_2$ values. Since mouth breathing is common in obese patients, especially those with a history of obstructive sleep apnoea, exhaled flow distribution between the mouth and nose highly affects the accuracy of capnometry. Capnography in spontaneously ventilating obese patients, therefore, is not the ideal technique for adequate haemodynamic monitoring. Capnography in these patients may be used to assess the global (changes in) haemodynamic status.

EXTENDED HAEMODYNAMIC MONITORING

Table 2 summarises further methods for haemodynamic monitoring.

Central venous catheterisation

CVP may be measured by single cannulation of the internal jugular, subclavian or axillary vein or (as part of) pulmonary artery catheterisation. Alternatively, peripherally inserted central catheters can be used in the obese to measure central venous pressure and to ensure reliable vascular access (infusion of fluids, intravenous medication, diagnostic blood draws).[34] The efficacy of central venous pressure measurements to judge intravascular volume status or fluid responsiveness remains controversial, both in obese and non-obese patients.[35]

Pulmonary artery catheter

The pulmonary artery catheter (PAC) has been used frequently in critically ill patients, after its introduction in clinical practice in the late 1970s.[36] The PAC provides haemodynamic data (i.e., pulmonary artery pressure, pulmonary capillary wedge pressure, central venous pressure and CO by thermodilution) to judge cardiovascular status and to assist therapeutic decisions.[37] An external pacemaker can be introduced via adjusted PAC systems in case of chronotropic incompetence or haemodynamically important bradyarrhythmias. Critically ill obese patients demonstrate elevated right atrial, mean pulmonary artery, and pulmonary artery wedge pressures. It should be noted that the validity of derived parameters indexed to body surface area has been questioned in morbidly obese patients. Several studies have demonstrated that these parameters are appropriate and valid when indexed to body surface area. The large body surface area in the obese patient, in fact, does not affect these measurements.[12,38]
Inappropriate use as well as poor interpretation of the PAC data may increase morbidity and mortality.[39-43] In a study involving patients including BMI >30 kg/m^2, there

Table 2. *Extended haemodynamic monitoring*

Monitoring device	Measurements / parameters	General remarks or problems	Remarks or problems in obese patients
Central venous catheterisation	Central venous pressure, waveform, respiratory variations Central venous blood gas analysis (pH, PcvO2, ScvO2, PcvCO2, Hb)	Locations for insertion: Internal jugular vein – Subclavian vein – Femoral vein	Femoral vein less favourable because of increased infection (intertrigo) and thrombosis risk in the obese
	Transpulmonary techniques (thermodilution or by indicator): Cardiac output – Stroke volume – Stroke volume variation – Intrathoracic blood volume – Extravascular lung water – Global end-diastolic volume – DO2	Transpulmonary technique may be inaccurate in case of heart valve dysfunction (e.g. tricuspid and/or aortic valve regurgitation)	Ultrasound guided central catheter placement is advocated
Pulmonary artery catheter	Central venous pressure Pulmonary artery pressure Pulmonary capillary wedge pressure Thermodilution CO SmvO2	CO estimation by PAC may be inaccurate in case of heart valve dysfunction (e.g. tricuspid and/or aortic valve regurgitation) PAC measurements less predictive with respect to global left ventricular function and preload conditions Option: application of external right ventricular pacemaker lead via PAC	Indexed haemodynamic measures are comparable for obese and non-obese
Carbon dioxide rebreathing technique (NICO)	CO (by Fick's principle)	Only possible in mechanically ventilated patients	Accurate CO measurement depends on dead space ventilation and pulmonary shunts CO2 administration in laparoscopic interventions may disturb measurements
Echocardiography – TTE – TEE	Cardiac output Flow parameters Anatomical data (dimensions, congenital defects and proximal great vessels) Ventricular systolic and diastolic function Heart valve anatomy and function Detection of myocardial ischaemia Pulmonary artery pressures Preload parameters Pericardial effusion Vegetation (Infective endocarditis) Neoplasm	Less suitable for continuous measurements TEE is advocated in case of (suspicion of) infective endocarditis TEE is not applicable in case of oesophageal / stomach diseases or severe coagulation disorders	Consider TEE in case of poor acoustic windows by TTE due to obesity
Oesophageal Doppler	Cardiac output Stroke volume Preload conditions Afterload conditions Cardiac contractility Stroke volume variation	CO measurement is highly depending on correct aortic diameter recording CO measurement is highly depending on proper positioning of Doppler probe	Indexed haemodynamic measures are comparable for obese and non-obese

CO = cardiac output; PAC = pulmonary artery catheter; TEE: transoesophageal echocardiography; TTE = transthoracic echocardiography.

was no significant difference in outcome in high-risk surgical patients monitored with PAC versus central venous catheter.[44] More importantly, a higher incidence of pulmonary embolism in the PAC group was reported. Not surprisingly, the use of PAC has decreased over the years, despite critical study results in favour of the PAC in selected patients treated by PAC-trained physicians.[45]

CO_2 rebreathing techniques

CO_2 rebreathing techniques can be used as a non-invasive method for calculating CO. CO_2 rebreathing as a technique is based on the differential Fick equation and is applicable in mechanically ventilated patients.[46] The precision of CO measurements in critically ill patients may vary by lack of haemodynamic stability, intrapulmonary shunting

and dead space ventilation.[47,48] Morbidly obese patients with pre-existing lung disease or postoperative atelectasis showed poor agreement of haemodynamic parameters derived from CO_2 rebreathing techniques when compared with the gold standard.[49] Newly developed equipment, algorithms and software have improved the performance of these techniques.[50] However, further developments and validations are needed.

Echocardiography

Echocardiography has proved to be of value in the management of patients with haemodynamic instability in the ICU as it may provide both anatomical and functional cardiovascular information.[51,52] Transthoracic echocardiography (TTE) is often severely limited in obese patients because of poor acoustic windows.[51] Dramatic improvements in ultrasound image quality have been achieved with the development of harmonic imaging.[53] Harmonic imaging exploits the formation of ultrasound signals that return to the transducer at a multiple of the transmitted (fundamental) frequency, referred to as the harmonic frequency. Other enhancements include the use of contrast agents capable of producing left ventricle cavity opacification from a venous injection to delineate the endocardial border. Several contrast agents are currently available that contain albumin microspheres filled with perfluorocarbon gas, allowing for passage of contrast through the lungs with appearance of contrast in the left ventricle.[54]

Transoesophageal echocardiography (TEE) can provide detailed information in most obese patients in contrast to transthoracic echocardiography.[51,52,55] TEE is particularly useful in critically ill patients with unexplained haemodynamic instability to rule out left and/or right ventricular failure, tamponade, hypovolaemia, and valvular dysfunction.[56,57] Moreover, the presence of multiple indwelling catheters, the need for parenteral nutrition, and prolonged mechanical ventilation increase the likelihood of bacteraemia and subsequent endocarditis. Because the classical clinical findings associated with endocarditis are uncommon or difficult to assess in obese patients, echocardiography may facilitate the diagnosis of endocarditis, as part of the diagnostic process (Duke's criteria).

Oesophageal Doppler

Pulsed Doppler techniques can be used to assess blood flow velocities. The oesophageal Doppler technique measures blood flow velocity in the descending aorta by means of a Doppler transducer (4 MHz continuous-wave, or 5 MHz pulsed-wave, according to manufacturers) placed at the tip of a flexible probe.[47,58] Oesophageal Doppler, in essence, provides almost continuous haemodynamic information. Oesophageal Doppler measurements, however, are very sensitive to probe movement. This implies that this method can only be used in patients who are not moving much (i.e. in operating room settings or in well-sedated ICU patients).

The algorithms used to calculate CO and SV vary slightly according to manufacturers and are highly influenced by correct aortic diameter estimation, as is calculated by R square. The estimation of stroke volume using oesophageal Doppler relies on the measurement of stroke distance in the descending aorta (= velocity-time integral), which is then converted into systemic stroke volume. Oesophageal Doppler has the possibility to estimate SV variations in time (i.e. stroke volume variation) which may be indicative of fluid responsiveness and may guide fluid balance in sepsis and trauma patients.[59]

FUTURE TECHNIQUES

Impedance techniques

Cardiac function may be non-invasively assessed by bio-impedance techniques. The theoretical basis for impedance cardiography is founded on Ohm's law, in which the thorax is considered a cylinder with two major components: a poorly conductive cylindrical static tissue impedance surrounding a high conductive cylindrical blood resistance.[60] After applying a constant, low-amplitude (0.5-4.0 mA), high-frequency (50-100 kHz), alternating electrical current to the thorax a voltage and time dependent induced voltage change can be measured. The voltage changes are used to calculate stroke volume, as well as CO, systemic vascular resistance and thoracic fluid content.

The accuracy of transthoracic bio-impedance technique depends on tissue, organ and fluid physics. These physical characteristics are summarised in the calculations by (assumed) constants. These assumptions are mostly derived from empirical geometric constructs in humans with average characteristics, including length and body weight.[60] The pathophysiological assumptions, however, may not hold true in the obese patient, thereby invalidating SV measurements (e.g. due to differences in aortic compliance and differences in intrathoracic volume and content).[60] The routine use of impedance cardiography for the measurement of CO in obese patients is still being questioned, despite attempts to improve the calculation algorithms.[60-63] Alternative forms of bio-impedance techniques are being developed to improve accuracy (e.g., endotracheal CO monitoring). Future studies are needed to prove clinical application and benefit of these techniques in the critical care setting, including the care of the morbidly obese patient.

Microcirculatory investigation

Apart from systemic haemodynamic consequences, the microcirculation may be impaired in many disease or

shock states.[64] The microcirculation comprises the blood flow and perfusion of vessels smaller than 100 microns. Evaluation and modification of the microcirculation by variable means (e.g. near infrared spectroscopy; Sidestream darkfield imaging; orthogonal polarisation spectral imaging; gastric tonometry, sublingual capnometry) is the subject of future investigation.[65] Whether this is relevant and/or beneficial in obese critically ill patients is still to be answered.

VASCULAR ACCESS

Central venous access may be challenging in obese patients once the traditional anatomical landmarks are not that clear or even lacking. The increased skin-blood distance and the short, stubby neck in morbidly obese patients may make internal jugular and subclavian venous cannulation even more difficult.[66] In line with this, more complications with central venous catheter placement are observed in obese patients (e.g. increased incidence of catheter malpositions and local puncture complications).[66] These complications are associated with lower experience of operators and higher numbers of needle passes.[67-70] In obese patients the number of skin punctures with catheter insertion attempts and delayed catheter changes have been shown to be related to more catheter-related infections and thrombosis.[67] With respect to infection prevention, femoral venous access may be less desirable or even impossible because of severe intertrigo in the obese.

Peripherally inserted central catheters can be used in the obese to measure central venous pressure and to ensure reliable vascular access.[34] Peripheral vascular access in morbidly obese patients, however, may be problematic and, for example, may necessitate placement of a central venous line for administration of medication intravenously or to facilitate regular blood draws for diagnostic purposes.

Two-dimensional ultrasound can be used to localise and define the anatomy of central veins. Colour Doppler may be of help to differentiate between veins and arteries by different characteristics in flow and collapsibility. Veins are usually larger in size, non-pulsatile, easily compressible and distended when the patient is placed with the head down or when the Valsalva manoeuvre is performed.[71] Real-time ultrasound guidance for cannulation of the internal jugular, femoral, or subclavian veins decreases the risk of failed cannulation, improves first success to catheter placement and facilitates faster placement compared with the landmark technique in morbidly obese patients.[68,72-74] According to current opinion, ultrasound-guided central venous catheterisation is strongly advocated in morbidly obese patients.[1,75] In both obese and non-obese patients, cannulation of peripheral veins and arteries may also be guided by ultrasound techniques but more studies are needed to prove its benefit.[76] Once in place, the central venous catheter should be cared for with upmost sterility, and insertion sites should be regularly checked for possible infection.

In case of life-threatening conditions (cardiopulmonary arrest, shock, sepsis, major trauma, extensive burns, status epilepticus) in which intravenous access cannot be obtained, intra-osseous access may be an alternative. Intra-osseous access is indicated in the critically ill patient of any age when rapid and timely intravascular access is needed. As in non-obese patients, the intra-osseous approach is suitable for morbidly obese patients with urgent conditions in whom multiple attempts at intravenous access have failed.[77]

CONCLUSIONS

Haemodynamic monitoring of morbidly obese patients in the ICU may be technically difficult. Because of the increased body weight, the ICU physician may be confronted with unique, challenging problems in morbidly obese patients. With respect to haemodynamic monitoring most aspects are quite equal between obese and non-obese patients. The clinician, however, should be aware of the basic pathophysiological principles of the applied monitoring tools. Its theoretical assumptions and calculations may be invalidated because of the high body weight in obese patients. When indexed for body surface area (e.g., CO) or predicted lean body weight (e.g., extravascular lung water) reliable haemodynamic data are comparable between obese and non-obese individuals. Morbid obesity, therefore, should not complicate the interpretation of haemodynamic data.

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REVIEW

Immunoparalysis in sepsis

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ABSTRACT

Although therapeutic opportunities in medicine continuously improve, death is inevitable in some cases due to limitations in treatment. When patients die without a conclusive diagnosis, autopsy studies can provide essential information in order to improve pathophysiological reasoning. We describe two patients who died after a prolonged course of sepsis and were diagnosed with the unsuspected presence of aspergillosis at autopsy. Literature review demonstrates that due to apoptosis and immunological interactions, septic patients become susceptible to opportunistic infections, a state described as immunoparalysis.

KEYWORDS

Aspergillosis, immunosuppression, sepsis, T cell differentiation

What was known about this topic?
Implementation of the Surviving Sepsis Guidelines has led to optimisation of patient care during the hyper-inflammatory phase of sepsis. Mortality in these patients, however, remains relatively high. Invasive aspergillosis is a severe fungal infection that affects immunocompromised patients, but is also described in intensive care patients with no prior history of immunosuppression.

What does this add?
These two cases demonstrate that a prolonged state of sepsis impairs cellular immune system functioning and predisposes patients to opportunistic infections. Disseminated invasive aspergillosis can develop without clinical suspicion and could be the cause of persistent multi-organ dysfunction syndrome. The immunological disturbances that have been described in recent studies may provide potential improvements in the therapeutic strategy for sepsis, but source control will remain a big issue.

INTRODUCTION

Sepsis embodies a cascade of systemic inflammatory responses that may progress to severe sepsis and septic shock. It is one of the leading causes of in-hospital death worldwide and represents 11-20% of all intensive care admissions.[1,2] Mortality increases with severity, and is approximately 46% for patients in septic shock.[3] In the Netherlands, sepsis represents approximately 3500 deaths annually.

The Surviving Sepsis Campaign has developed a resuscitation and management bundle in order to standardise patient care in the initial hours after admission.[4] National and international studies have demonstrated that implementation of these bundles significantly reduces mortality.[2,5,6] However, even when optimal support is provided, mortality remains relatively high.

CASE REPORTS

A 67-year-old patient with a medical history of chronic obstructive pulmonary disease and paroxysmal atrial fibrillation presented to the emergency department with complaints of melaena. Gastroscopy revealed an intramucosal adenocarcinoma of the pyloric antrum, for which he underwent subtotal gastric resection. The postoperative course was complicated with persistent fever and haemodynamic instability. Imaging studies revealed anastomotic leakage, for which surgical reconstruction was performed. Blood cultures were positive for *Streptococcus milleri, Candida albicans* and *Enterococcus faecium* successively. Although sputum cultures once showed

growth of *Aspergillus species*, Galactomannan enzyme immunoassay was not performed. The patient's condition did not improve and leakage of pancreatic secretion caused recurrent gastrointestinal blood loss. CT scan of the thorax revealed atelectasis of the left lower lobe with mild pleural effusion and persistent abdominal fluid collections. Despite repetitive surgical interventions and extensive antibiotic and antifungal treatment, the patient remained septic and eventually died due to multiple organ dysfunction. Autopsy revealed the unsuspected presence of aspergilloma in the respiratory tract with systemic activity and metastatic growth in the heart and kidneys (*figure 1*).

Two years earlier, a 52-year-old patient with no relevant medical history had developed a similar course after surgical resection of a perforated ileum. Postoperatively, she remained dependent on ventilatory and haemodynamic support. Multiple surgical interventions were performed in order to correct anastomotic leakage, but septic shock persisted. Blood cultures revealed sequential growth of *Pseudomonas aeruginosa, Enterococcus faecium* and *Candida albicans*. Thoracic CT scan showed ground glass opacity in the basal fields bilaterally and cystic bronchiectasis in the right lower lobe. As in the case above, *Aspergillus fumigates* was grown once in the sputum but Galactomannan enzyme immunoassay was not performed. Eventually, the patient developed a severe encephalopathy and although extensive antibiotic treatment was administered, she remained in a depressed state of consciousness. CT cerebrum suggested haemorrhagic lesions in the frontal lobe (*figure 2*). Consecutive electroencephalography did not reveal improvement in cerebral performance and therefore multidisciplinary consultation concluded to discontinue supportive treatment. At autopsy, vaso-invasive growth of *Aspergillus* was found bilaterally in the lungs, with systemic mycotic emboli and metastatic involvement of the cerebrum.

Figure 1. *Growth of Aspergillus in the myocardium*

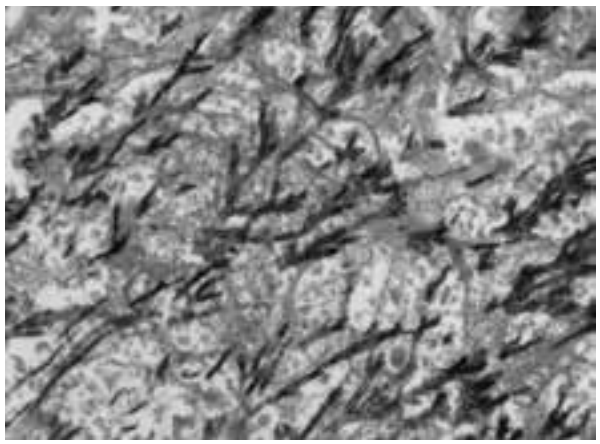
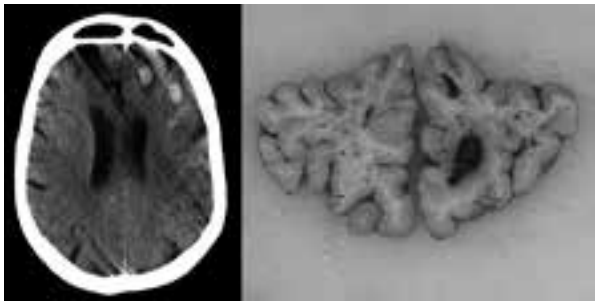


Figure 2. *Cerebral lesions on CT scan were classified as metastates of invasive aspergillosis at autopsy*

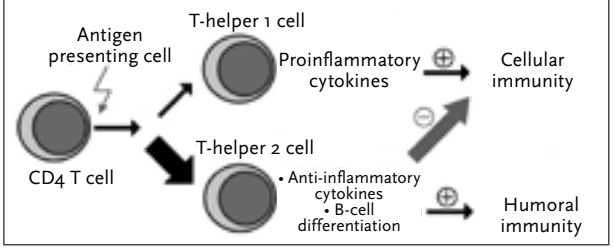


DISCUSSION

As these two cases evidently demonstrate, opportunistic infections may develop in critically ill patients with no history of immune suppression. Invasive aspergillosis is a life-threatening infection that primarily affects patients with haematological malignancies, autoimmune diseases, AIDS or immunosuppressive therapy. Diagnosis is difficult to obtain, since it requires presence of *Aspergillus* in the histopathological analysis of sterile tissue specimens. In the last decade, several case series have described invasive aspergillosis in intensive care patients without a history of immune suppression.[7,8] It seems multi-organ dysfunction syndrome predisposes patients to opportunistic infections. In 1995, Ertel *et al*. demonstrated that the release of pro-inflammatory cytokines in whole blood was significantly impaired in septic patients compared with control patients without infection.[9] Autopsy studies later revealed profound apoptosis of B cells, CD4 T cells and follicular dendritic cells in patients who died of sepsis.[10] The apoptosis of these cells appears to play a key role in the pathophysiology of immune suppression.

A decrease in the absolute number of circulating lymphocytes and dendritic cells obviously reduces the functioning of the immune system. Furthermore, presence of apoptotic lymphocytes substantially increases anti-inflammatory cytokine production and impairs the release of pro-inflammatory cytokines (IL-10 and TNF-α respectively).[11] The production of these cytokines is directed by CD4 T cells, which are activated by antigen-presenting cells and can either proliferate to T-helper 1 or T-helper 2 cells. The presence of T-helper 1 cells stimulates cytotoxic T cells and thereby increases cellular immunity, whereas T-helper 2 cells stimulate humoral immunity and simultaneously provide negative feedback to the cellular immune system. After ingestion of apoptotic cells, antigen-presenting cells predominantly stimulate the proliferation of CD4 T cells in T-helper 2 cells and cause an overshoot in negative feedback to cellular immunity (*figure 3*).[10,12]

Figure 3. *Presence of apoptotic cells stimulates proliferation of CD4 T cells into T-helper 2 cells and thereby causes an overshoot in negative feedback to cellular immunity*



Since the cellular immune system is crucial for protection against opportunistic infections, patients become less competent in eliminating these opportunists. Lastly, the presence of apoptotic cells seems to accelerate lymphocyte tolerance to pathogens, a mechanism described as anergy.[12] The combination of these pathways causes cellular immune system paralysis.

In conclusion, the cascade of sepsis does not end at the stage of septic shock. Immune responses in sepsis are biphasic; the initial hyper-inflammatory phase is followed by anti-inflammatory reactions that induce immunoparalysis. During sepsis, patients with prior normal immune system functioning become susceptible to opportunistic infections such as invasive aspergillosis. This may partially explain the mortality that persists despite the implementation of the Surviving Sepsis Guidelines. Further insight into the immunological disturbances in septic patients may lead to the development of immunomodulatory therapy. Currently, several potentially beneficial immunosupportive agents are being studied in clinical trials.[13,14]

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ORIGINAL ARTICLE

Venous thromboembolism and prothrombotic parameters in Klippel-Trenaunay syndrome

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ABSTRACT

Background: In Klippel-Trenaunay syndrome (KTS), a congenital combined vascular (capillary, venous and lymphatic) malformation with localised disturbed growth, venous thromboembolisms (VTEs) are frequently reported in small cohorts.

Design and methods: We quantified the frequency of VTE by screening a large KTS-patient cohort with duplex compression ultrasonography. Additionally, we performed a case-control study to evaluate whether coagulation alterations were related to VTE and magnitude of vascular malformations as quantified by magnetic resonance imaging (MRI).

Results: Twenty-nine (39%) of 75 patients had signs of current or previous VTE, including superficial venous thrombosis, six (8%) of whom had a deep venous thrombosis or a pulmonary embolism. Compared with 105 controls, 54 adult patients (both: median age 33 years) had higher plasma levels of D-dimer, medians 266 (IQR 195-366) versus 457 (IQR 270-3840) µg/l (p<0.001), respectively. They tended to have lower protein C (p=0.10) and free protein S (p=0.07) levels compared with controls. Compared with young-adult controls (n=62), KTS children (n=21) also had higher median D-dimer levels (p<0.001), and lower protein C (p=0.003) and protein S (p=0.01) levels. The extent of the vascular malformations on MRI was positively correlated with D-dimer plasma levels (r-spearman=0.329; p<0.05).

Conclusions: Otherwise healthy KTS patients had a very high rate of current or previous VTE. Very high D-dimer levels were observed and these were related to the extent of the vascular malformation. Based on these findings we advise appropriate patient education on the signs and symptoms of thromboembolic complications in these patients, and screening for VTE in case of complaints.

KEYWORDS

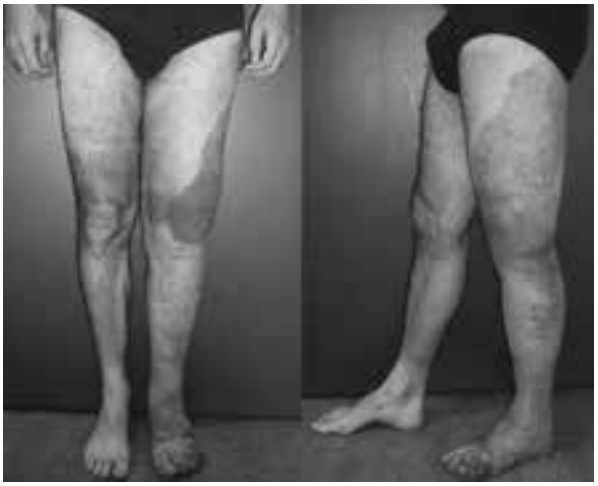
Venous thromboembolism, Klippel-Trenaunay syndrome, vascular malformation, duplex compression ultrasonography, deep venous thrombosis, D-dimer.

INTRODUCTION

Klippel-Trenaunay syndrome (KTS) is a congenital malformation syndrome characterised by a combination of capillary, venous and lymphatic malformations with a localised disturbed growth of bone and/or soft tissue (*figure 1*).[1] Clinical presentation may vary from being asymptomatic to developing potentially life-threatening complications, such as deep vein thrombosis (DVT), pulmonary embolism (PE), and recurrent bleeding.[2-5]

Although not investigated systematically, venous thromboembolism (VTE) has been reported to occur in 8-22% of KTS patients.[2] Despite the fact that over 30 case reports on KTS-associated pulmonary embolism have been published,[2,3,5-17] including alarming cases of belated

Figure 1. *A patient with Klippel-Trenaunay syndrome affecting the left leg*



Combined capillary, venous, and lymphatic malformation, with hypotrophy in the length and hypertrophy in the girth.

diagnosed recurrent pulmonary embolism or pulmonary hypertension,[3,5,8-12] the exact mechanism underlying the hypercoagulability in vascular malformations remains unclear. However, blood stagnation within the distorted, enlarged venous blood vessels may lead to coagulation activation,[18] though this has never been studied in a large KTS group.[1]

Therefore, the aims of the present study were to investigate the prevalence of present or previous thrombotic complications in KTS patients, to identify coagulation alterations compared with matched controls and to determine whether these changes were present at a young age.

DESIGN AND METHODS

Patients and procedures

All patients from a well-characterised Dutch cohort of KTS patients were asked to participate in the study and, if so, gave written informed consent. The patients were acquired from the Dutch KTS support group, and the multidisciplinary teams dedicated to vascular malformations in two Academic Medical Centres, between May 2006 and May 2007. Diagnosis of KTS was based on a combination of capillary, venous, and lymphatic malformations plus disturbed growth regulation, as previously described.[19] Results on the prevalence of chronic thromboembolic pulmonary hypertension in a subset of this group were published previously.[20] Plasma samples from two matched controls (on age, gender and use of oral anticontraceptives) were recruited from healthy volunteers in the AMC. Because the Medical Ethics Committee deemed it unethical to involve healthy children in this study, young adults were used as controls for the paediatric patients. The protocol was approved by the Medical Ethics Committee of the AMC, and carried out in accordance with the principles of the Declaration of Helsinki.

History taking, physical examination and duplex ultrasonography

Data regarding history of clinically manifest haemorrhages, and venous thromboembolism were collected. When deep venous thrombosis or pulmonary embolism was reported, we confirmed whether the diagnosis was established using an objective test (either compression ultrasonography, venography, ventilation perfusion scan or multi-slice CT). All patients were physically examined, looking for signs of haemorrhages or venous thromboembolism.

Flow characteristics, sub and clinically manifest superficial or deep venous thrombosis were obtained by duplex ultrasonography of the affected limb, performed by two experienced sonographers. With this procedure, the entire deep venous system was imaged from the groin or shoulder down to the distal system in the calf or arm. The only criterion accepted for a diagnosis of superficial or deep venous thrombosis was the finding of one or more non-compressible superficial or deep venous segments.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) studies were performed to delineate the extent of the vascular malformation in case of suspicion of multiple muscle and/or bone involvement, when disabling pain existed, before therapeutic intervention, or for other reasons. Because of the need for general anaesthesia, children (≤12 years) were only investigated by MRI when therapeutic intervention was considered. We recorded the anatomic location of the vascular malformation, and the extent was categorised in groups of increasing severity. The least affected group of patients had slight evidence of vascular malformations on MRI scanning (muscle atrophy, alterations in fat tissue, varicose veins). The second group had only (sub) cutaneous vascular lesions. The third group had (sub) cutaneous lesions combined with lesions in another tissue compartment (muscle or osseous involvement). The patients with extensive (three or more affected muscles) disease or a combination of muscle and osseous involvement on MRI scans were included in the last group.

Reagents and assays

Blood samples were collected into citrated tubes (Becton Dickinson, San Jose, CA), and centrifuged within 30 minutes for 20 minutes at 1700x g and 15°C, repeated for 15 minutes at 3000x g and 15°C. Plasma was stored at -80°C. Activated partial thromboplastin time (APTT) and prothrombin time were performed on a Behring

Coagulation System with reagents from the manufacturer (Siemens Healthcare Diagnostics). The fibrinogen concentration was derived from the change in optical signal during the prothrombin time determination. The plasma concentrations of prothrombin fragment 1+2 (F1+2) were measured by ELISA (Siemens Healthcare Diagnostics). D-dimer levels were determined by a quantitative ELISA method (Asserachrom® D-dimer, Diagnostica Stago, Asnières, France).

Plasmin-antiplasmin (PAP) complexes were determined with an ELISA from DRG (Marburg, Germany). The endogenous thrombin potential (ETP) was determined with a Calibrated Automated Thrombogram®. As this assay was employed to further explore the aetiology of the D-dimer elevation, it was only performed on the two subgroups of patients in the highest and lowest D-dimer tertile. The Calibrated Automated Thrombogram® assays the generation of thrombin in clotting plasma using a microtiter plate reading fluorometer (Fluoroskan Ascent, ThermoLab systems, Helsinki, Finland) and Thrombinoscope® software (Thrombinoscope BV, Maastricht, the Netherlands). The assay was carried out as described by Hemker *et al.*[21] and the Thrombinoscope manual. Coagulation was triggered by recalcification in the presence of 5 pM recombinant human tissue factor (Innovin®, Siemens Healthcare Diagnostics), 4 μM phospholipids, and 417 μM fluorogenic substrate Z-Gly-Gly-Arg-AMC (Bachem, Bubendorf, Switzerland). The endogenous thrombin potential (ETP) and related parameters were calculated using the Thrombinoscope software. Four parameters were derived from the thrombin generation curve: lag time, peak height, peak time and ETP (area under the curve).

Statistical analysis

Statistical analysis was performed by using SPSS 16.0.2 (SPSS, UK). All numbers are medians with corresponding ranges, unless stated otherwise. The Mann-Whitney *U*-test was used for non-parametric numerical data. Differences between groups of categorical data were tested with the Chi-square (χ^2) test. To investigate whether high or low levels of coagulation parameters were more prevalent among KTS patients we calculated odds ratios (OR) for values above the 75th and 90th percentile of the control group, or 25th and 10th percentile of the control group, with their corresponding 95% confidence intervals (95% CI).

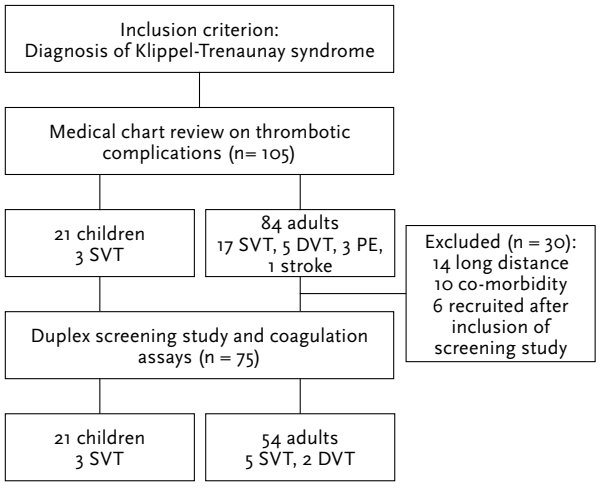
RESULTS

Patients

In total 105 (61 women, 58%) patients were eligible for inclusion, of which 30 patients did not participate. The reasons for exclusion are depicted in *figure 2*. The mean age of the non-participants was 30 years (range 5-68 years), 57% were female. Four of these patients had a history of venous thromboembolism (two had proven deep vein thrombosis, one had recurrent pulmonary embolism, and one had both), and another suffered from an ischemic stroke. Two patients, reported earlier,[5] died due to chronic thromboembolic pulmonary hypertension, and were therefore not amongst these 105 patients.

Data of 75 cases were available for the coagulation analyses. Characteristics of patients are listed in *table 1*. The cases were compared with 105 healthy adult controls: 63% women, with a median age of 33 (range 22-66) years.

Figure 2. *Flowchart of inclusion of KTS patients*



In total 105 (61 women, 58%) patients were included in the medical history part of the study. Thirty patients did not participate in the subsequent duplex ultrasonography screening and blood withdrawal part of the study. Reasons for their exclusions are listed in the graph. Ten patients were excluded because of invalidating comorbidity or symptoms. These symptoms were extensive pain (n=5), immobility (n=3) and shortness of breath due to recurrent pulmonary embolism (n=2). SVT = superficial venous thrombosis; DVT = deep venous thrombosis; PE = pulmonary embolism.

Venous thromboembolism: medical history and duplex ultrasonography

Medical history regarding proven venous thromboembolism revealed three patients (4%) with a previously documented deep venous thrombosis, one patient with a previous pulmonary embolism (1.3%) and 24 (32%) with previous superficial venous thrombosis. The pulmonary embolism was diagnosed in a patient who had just given birth to a child and imaging also showed a deep venous thrombosis.

A total of 70 patients underwent duplex ultrasonography. Of the five patients without ultrasonography, one was too young (<1 year), three refused and one had his affected leg amputated because of painful chronic infections. Thirty-five patients had venous anomalies: absent or hypoplastic deep venous system (n=6), hyperplastic deep

Table 1. *Baseline clinical characteristics of all the patients*

Characteristic	n=75
Age (years (range))	24 (1-77)
Female sex	44 (58.7)
Localisation	
- One upper limb	7 (9.3)
- One lower limb	51 (68)
- Both lower limbs	7 (9.3)
- All limbs, thorax and face	7 (9.3)
- Trunk	2 (2.7)
- Gluteus	1 (1.3)
Risk factors for venous thrombosis	
- Previous VTE	25 (33.3)
Superficial vein thrombosis	24 (32)
Deep vein thrombosis	3 (4)
Pulmonary embolism	1 (1.3)
- Immobilisation (walking aids)	1 (1.3)
- Recent surgery (<3 months)	0 (0)
- Oral contraceptives (women ≥18 only)	8 (17.6)
- Pregnancy	0 (0)
- Known cancer	0 (0)
Protective measures	
- Using anticoagulant medication	
Vitamin K antagonists	0 (0)
NSAIDs	7 (3.8)
Acetylic acid	4 (5.3)
- Use of therapeutic elastic stockings	42 (56)

Numbers are followed by the percentage of patients. NSAIDs, non-steroidal anti-inflammatory drugs.

venous system (n=2), double inferior caval vein (n=5), marginal lateral vein (n=12), abnormalities in great or small saphenous veins (n=16), and a double deep femoral artery (n=1).

Superficial venous thrombosis was observed in nine (12.7%) cases, while one case (1.4%) had evidence for a previously unknown superficial venous thrombosis. One patient appeared to have a current deep venous thrombosis and another patient showed an older previously unknown thrombosis in the common femoral vein, external iliac and common iliac vein.

When combining the results of duplex ultrasonography and medical history, 29 out of 75 patients with KTS (39%) had ever experienced a thromboembolic complication, six (8%) of whom had either a deep venous thrombosis or pulmonary embolism.

Coagulation parameters

The levels of D-dimer and PAP complexes in the adult KTS group were higher compared with those in the control group; very high D-dimer levels were observed in a number of patients (*table 2*). The D-dimer levels were also high in patients without signs of current superficial or deep venous thrombosis, median 407 (IQR 236-884). The odds ratio for a D-dimer value above the 75th percentile of the control group was 4.8 (95% CI 2.3-10.1) and above the 90th percentile 4.4 (95% CI 1.6-11.9). There was also a trend to lower median levels of the natural anticoagulants protein C (p=0.10) and free protein S (p=0.07) in cases compared with controls. There were significantly more cases with a protein C value below the 25th percentile of the control group (odds ratio 2.5, 95% CI 1.2-5.2), and below the 10th percentile (odds ratio 3.3, 95% CI 1.1-9.8). Comparing the children with KTS to young-adults we generally observed the same trend (*table 2*). The D-dimer levels were higher (p<0.001) and the median levels of protein C (p=0.003) and protein

Table 2. *Results on markers of coagulation activation: adult cases versus adult controls and children with KTS versus young-adult controls*

	Adult cases (n=54)	Adult controls (n=105)	P-value*	Child cases (n=21)	Young-adult controls (n=62)	P-value*
Age	33 (23-45)	33 (28-44)	0.32	9 (4-12)	28 (26-31)	<0.001
Sex (M:F)	20:34	39:66	0.99	11:10	26:36	0.41
Prothrombin time (sec)	12.5 (11.9-13.0)	12.4 (12.0-12.8)	0.76	12.6 (12.3-12.9)	12.5 (12.2-12.9)	0.55
Activated partial thromboplastin time (sec)	31.9 (29-34.6)	32.0 (30.1-35)	0.25	33.0 (31.4-35.6)	32.8 (30.1-35.5)	0.39
Markers of coagulation activation						
- Fibrinogen (g/l)	3.1 (2.3-3.7)	2.9 (2.4-3.4)	0.29	3.0 (2.7-3.4)	2.9 (2.4-3.3)	0.20
- Prothrombin fragment 1+2 (pmol/l)	203 (127-304)	209 (163-268)	0.76	166 (118-220)	184 (149-218)	0.38
- Von Willebrand factor (%)	97 (84-134)	100 (76-118)	0.41	87 (69-117)	97 (77-115)	0.48
- D-dimer (µg/l)	457 (270-3840)	266 (195-366)	<0.001	346 (298-771)	235 (168-338)	<0.001
- Plasmin-antiplasmin complexes (µg/l)	514 (373-758)	382 (300-476)	<0.001	405 (306-480)	384 (299-479)	0.70
Anticoagulant assays (% of normal)						
- Protein C	104 (90-123)	110 (99-130)	0.10	95 (84-107)	108 (96-124)	0.003
- Total Protein S	105 (91-122)	103 (93-123)	0.63	104 (95-118)	99 (88-118)	0.39
- Free Protein S	94 (83-105)	101 (87-111)	0.07	89 (78-96)	101 (84-110)	0.01
- Antithrombin	102 (96-108)	102 (96-108)	0.80	106 (99-110)	101 (96-107)	0.06

Numbers are medians (interquartile range). *Mann-Whitney test or Chi-square-test when applicable.

S (p=0.01) were lower in this group of young patients compared with young-adult controls.

Extent of malformations and D-dimer levels

Lesions of 40 KTS patients were examined by MRI. Of the 35 patients without an MRI scan, 20 did not have any symptoms, one had his leg amputated, 14 were too young and without a medical indication. Thirty-seven MRI scans could be evaluated. Three patients were in the least affected group on MRI. Ten patients had only (sub)cutaneous involvement on MRI. Ten patients had (sub)cutaneous involvement combined with another tissue compartment (muscles or osseous involvement). Fourteen patients had extensive disease with at least three affected muscles or a combination of muscle and osseous involvement on MRI. The median D-dimer levels in these four groups were 315 µg/l, 364 µg/l, 424 µg/l, and 975 µg/l respectively. The extent of the vascular malformations as quantified by MRI was significantly positively correlated with the D-dimer plasma levels (r-spearman=0.329; p<0.05).

High D-dimer versus low D-dimer

To further explore the striking high D-dimer results, we determined the endogenous thrombin potential. We divided KTS patients in tertiles according to plasma levels of D-dimer, and compared the highest with the lowest tertile (*table 3*).

PAP complexes were clearly higher in the highest tertile, while prothrombin fragment F1+2 and Von Willebrand factor were somewhat higher. Only subtle alterations in components of the endogenous thrombin potential and in protein C levels were observed (*table 3*).

DISCUSSION

The primary aim of this study was to estimate the prevalence of venous thromboembolism in the Dutch KTS group. In total, 39% of the patients had ever experienced thromboembolic complications, including superficial thrombosis. Of all patients, 8% had either a deep venous thrombosis or a pulmonary embolism. This is extremely high compared with the incidence rate of 1.9 per 10,000 person-years in adults aged 30-34 years in the general population.[22] Furthermore, a considerable number of patients had clinically silent thrombosis detected by ultrasonography.

Our second aim was to identify coagulation alterations in patients with KTS compared with matched, healthy controls. We observed high, sometimes extremely high, D-dimer levels, higher PAP complexes and somewhat lower protein C levels among the adult patients compared with controls. Among the children with KTS also the free protein S was lower. The variables, fibrinogen, F1+2, Von Willebrand factor, antithrombin and total protein S, did not differ between patients and controls.

D-dimer, which is a reflection of fibrin degradation, has gained a prominent role in the diagnostic work-up of patients with suspected thromboembolism. The prothrombin fragment F1+2 directly reflects thrombin

Table 3. *Endogenous thrombin potential and natural anticoagulants: cases in the highest D-dimer tertile versus lowest D-dimer tertile*

	Highest (n=25)*	Lowest (n=25)	P-value**
D-dimer (µg/l)	4417 (1041-7916)	234 (210-273)	n.a.
Thrombin generation			
- ETP (nM.min)	1530 (1355-1708)	1527 (1358-1656)	0.070
- Lag time (min)	2.67 (2.33-2.95)	2.29 (2.04-2.67)	0.023
- Peak (nM)	276 (260-313)	315 (282-336)	0.016
- Peak time (min)	5.17 (4.85-5.62)	4.67 (4.22-5.33)	0.018
Anticoagulant activity assays (% of normal)			
- Protein C	100 (86-114)	104 (93-122)	0.022
- Free Protein S	94 (83-101)	85 (78-104)	0.91
- Antithrombin	101 (96-105)	106 (100-112)	0.013
Prothrombin time (sec)	12.8 (12.1-13.4)	12.3 (12-12.9)	0.24
Activated partial thromboplastin time (sec)	34.0 (31.2-35.3)	31.4 (29-33.5)	0.065
Markers of coagulation activation			
- Fibrinogen (g/l)	2.9 (2.2-3.5)	2.8 (2.5-3.4)	0.52
- Prothrombin fragment 1+2 (pmol/l)	277 (182-473)	168 (106-213)	0.001
- Von Willebrand factor (%)	105 (93-141)	84 (63-106)	0.006
- Plasmin-antiplasmin complexes (µg/l)	730 (438-1174)	397 (287-501)	<0.001

Numbers are medians (interquartile range). *Cases were selected upon D-dimer level. In this table cases in the tertile with the highest plasma level of D-dimer are shown and compared to the healthy controls. **Mann-Whitney test.

generation, since this fragment is generated during the conversion of prothrombin to thrombin. Consequently, F1+2 may be a more specific test for coagulation activation than D-dimer. However, large observational studies have shown that in clinical practice D-dimer is a much better predictor of thromboembolism in patients than other assays reflecting coagulation activation or fibrinolysis.[23] Based on the significant difference in D-dimer, PAP complexes, and protein C, our results indicate that patients with KTS have a prothrombotic state.

To further analyse the finding that a considerable amount of patients had very high D-dimer levels, we determined the capacity of their plasma to form thrombin with the so-called endogenous thrombin potential. We found only subtle alterations in components of the endogenous thrombin potential in the high D-dimer tertile. This is accompanied by a strong increase in PAP complexes and F1+2, lower protein C and a trend towards a higher APTT. This indicates that the observed changes in D-dimers and higher rate of thrombotic complications is probably not caused by an inherent increased potential to generate thrombin of patient plasma. In contrast, we suggest that the venous malformations cause disruption of blood flow thereby initiating continuous generation of thrombin with concomitant consumption of coagulation factors activation of the fibrinolytic system.[24] The minor increase of Von Willebrand in the highest D-dimer tertile indicates that endothelial cell activation is probably not the explanation for the high D-dimer levels.

Though the exact mechanism behind hypercoagulability in vascular malformations remains to be elucidated, blood stagnation or altered flow within the distorted, enlarged venous blood vessels may be responsible.[18] Insufficient calf function has been suggested as a cause, but foot volumetry in KTS compared with controls showed no significant decrease in calf function in KTS patients.[2] Another explanation for the higher incidence of deep venous thrombosis can be abnormalities in the venous vessel walls, although plethysmography was unable to demonstrate this.[25] A disturbed coagulation system has been proposed as explanation for venous thromboembolism in KTS.[25] Patients with other large venous malformations showed serological evidence of coagulopathy, defined by decreased fibrinogen levels, elevated D-dimer, and prolonged prothrombin times, with normal to moderately low platelet counts.[26-28] The results in these studies are in line with the increase in lag time, time to peak, low peak thrombin, low protein C, and strongly elevated D-dimer in our patients. The severity of the coagulopathy reported in other venous malformations seemed to be correlated with the extent of the malformation.[26,28,29] We also showed a correlation between the extent of the malformation and the level of D-dimer. Mazoyer[26,28] proposed 'localised intravascular coagulation', or LIC, to be the cause of the coagulopathy in venous malformations. In our patients there are indications for some consumption of coagulation factors, but there is no clear consumptive coagulopathy.

This study has some limitations. First, ten patients did not participate in this study because they had high morbidity, e.g. extensive pain, recurrent pulmonary embolism, or were less ambulant because of great discrepancy in leg length and distal femur amputation due to KTS. Since most severe cases were not included, and two patients with known chronic thromboembolic pulmonary hypertension died before this study, we are certain that the incidence of deep vein thrombosis and pulmonary embolism presented in this study is not an overestimation of the prevalence of venous thromboembolism in this patient group.

Secondly, we were not allowed to recruit a matched control group for children younger than 18 years and had to compare them with young adults. However, the most important changes in plasma levels of proteins involved in coagulation cascade, i.e. D-dimer, protein C and free protein S, were not different between adults and children with KTS.

In conclusion, in this large cross-sectional cohort study of apparently healthy and young KTS patients, deep and superficial venous thrombosis is very common and a high proportion of these patients have high or extremely high D-dimer levels while there is only limited consumption of coagulation factors. Hence the presumed prothrombotic local effects in KTS are most likely down-regulated by a highly active fibrinolytic system.

Based on these findings we advise appropriate patient education on the signs and symptoms of thromboembolic complications in these patients. In case of symptoms, a low threshold for diagnostic testing for venous thromboembolism is necessary. Lifelong anticoagulation therapy should be considered in the case of a first deep venous thrombosis or pulmonary embolism, and venous thromboembolism prophylaxis should be directed to those KTS patients with hypercoagulability when they are exposed to one of the risk factors for venous thromboembolism, such as surgery, trauma or pregnancy.

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CASE REPORT

Unexpected symptoms after rhTSH administration due to occult thyroid carcinoma metastasis

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ABSTRACT

^{18}F-fluorodeoxyglucose positron emission tomography (^{18}FDG-PET) scintigraphy is a useful imaging technique in the evaluation of metastasised thyroid carcinoma. Administration of recombinant human thyrotropin (rhTSH, Thyrogen®) increases the diagnostic yield of this procedure. Here we present a 64-year-old male who was followed for Hürthle cell carcinoma of the thyroid with several intrapulmonary metastases. He developed sudden complaints of neck pain following rhTSH administration as part of the routine preparation for a diagnostic ^{18}FDG-PET/CT procedure. This investigation subsequently revealed a previously undetected metastatic lesion in the first cervical vertebra, with no signs of spinal cord compression. Treatment with a nonsteroidal anti-inflammatory drug reduced the symptoms sufficiently, and a few weeks later the neurosurgeon performed a complete resection of the metastasis. It is likely that the symptoms were caused by oedema and/or increased blood flow to the lesion. Physicians should be aware that rhTSH administration to patients with disseminated thyroid carcinoma may lead to sudden onset of symptoms caused by previously occult metastases.

KEYWORDS

Carcinoma, rhTSH, metastasis, side effects, surgery

INTRODUCTION

Generally, patients with differentiated thyroid carcinoma have a good prognosis, although the number of recurrences – within the 20% range – remains relatively high. In patients in whom the clinical symptoms or elevated serum thyroglobulin (Tg) levels suggest such disease recurrence, fluorine-18-labelled 2-fluoro-2-deoxy-D-glucose (^{18}FDG) positron emission tomography (PET) is often used to localise metastatic lesions. Already in 2002 our group and others showed that ^{18}FDG-PET during TSH stimulation was superior to ^{18}FDG-PET during thyroxin replacement and/or TSH suppression.[1,2] Several other reports and a recent meta-analysis have confirmed the added value of TSH stimulation, which leads to an increase in both the number of patients with ^{18}FDG-PET true-positive lesions and the number of detected lesions.[3] The application of ^{18}FDG-PET scans after recombinant human thyrotropin (rhTSH) administration can significantly alter clinical management,[3,4] and diagnostic sensitivity can also be improved by combining PET with a computed tomography (CT) scan.[5,6]

TSH stimulation in these patients can be achieved by means of either thyroid hormone withdrawal or administration of rhTSH (Thyrogen®). When there is a suspicion that metastases are localised in or near vital structures such as the spinal cord or cerebrum, thyroid hormone withdrawal may lead to prolonged TSH increase and subsequent stimulation of tumour growth, leading to neurological signs and symptoms. rhTSH is therefore often used to decrease the TSH exposure time. Although rhTSH administration is generally well tolerated, with minimal side effects, there have been reports of side effects in subjects with cerebral or spinal cord metastases.[7] Here we report a patient who, following rhTSH administration, developed complaints of neck pain due to a previously unknown metastatic lesion in the first cervical vertebra.

CASE

Our patient, born in 1947, was healthy until 2005 when he underwent a diagnostic hemithyroidectomy because of a 5 cm mass in the left thyroid lobe. Histological examination revealed Hürthle cell carcinoma. Subsequent treatment consisted of total thyroidectomy followed by ablation with 150 mCi radioactive iodine (^{131}I). A small thyroid remnant was seen on the post-ablation whole-body scan performed after ten days. Six months later, he received a second dose of 150 mCi ^{131}I. During thyroid hormone withdrawal, serum thyroglobulin (Tg) levels were 2.8 ng/ml (desired level <1.0 ng/ml) and serum was negative for anti-Tg antibodies. Post-therapeutic whole-body scintigraphy showed no abnormalities, and no signs of metastases were seen upon additional ^{124}I scintigraphy and magnetic resonance

Imaging (MRI) of chest and mediastinum

Subsequent treatment consisted of suppressive therapy with levothyroxine, and serum Tg levels decreased to 0.22 ng/ml. During the second half of 2007, serum Tg levels gradually increased to 6.2 ng/ml. rhTSH-stimulated ^{18}FDG-PET scintigraphy was therefore performed, revealing two small intrapulmonary metastases with tracer accumulation. A CT scan revealed multiple small (2-7 mm) intrapulmonary nodules suggestive of metastases. In the years that followed, his chest X-ray showed a gradual, albeit very slow, increase in the number and size of the intrapulmonary metastases, and serum Tg levels gradually increased. He experienced no health problems, and was able to exercise and continue all work activities without symptoms.

In the fall of 2011, we performed more extensive staging in order to assess the extent of tumour spread in addition to the pulmonary metastases, and to evaluate the possibility of repeated ^{131}I treatment. One day after administration of two intramuscular injections of 0.9 mg rhTSH, 24 hours apart, the patient developed severe localised neck pain, and was unable to rotate his neck. There were no signs of spinal cord compression. ^{18}FDG-PET with a CT scan revealed –in addition to the known intrapulmonary metastases – a large metastasis in the arch of the first cervical vertebra, with evident bone destruction, but with no compression of the spinal cord. He was treated with high doses of nonsteroidal anti-inflammatory drugs (NSAIDs), which reduced his symptoms, although he remained incapable of rotating his neck for at least 5-6 days. The pain gradually subsided, and he was able to stop NSAID treatment after 2-3 weeks. Four weeks later, the neurosurgeon performed a laminectomy and the metastasis was completely removed. This procedure went without significant blood loss and

Figure 1. *Total body ^{18}FDG-PET scintigraphy revealing multiple intrapulmonary metastases, as well as a metastasis on the left side in the neck, in the cervical vertebral column*

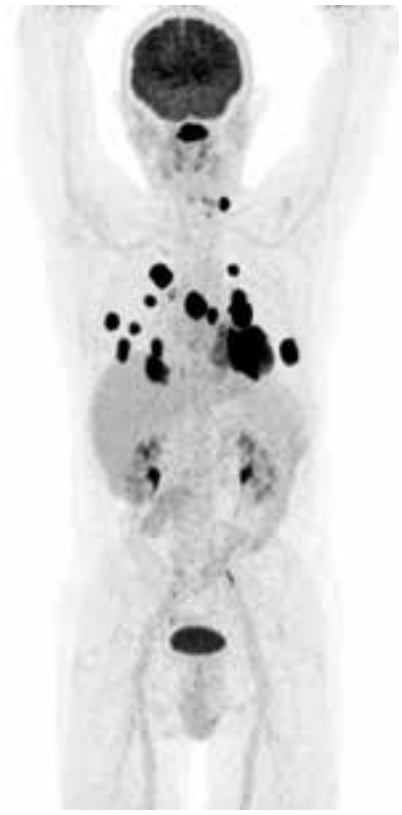
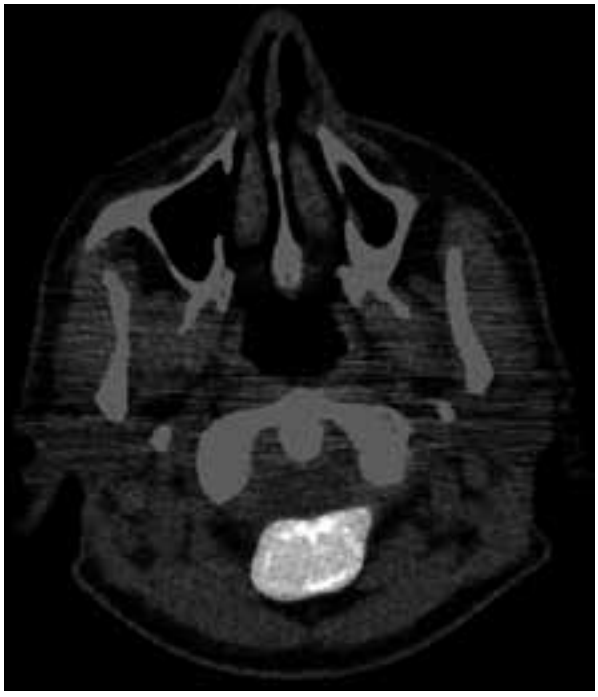


Figure 2. *Fusion of CT scan and ^{18}FDG-PET scan revealing a metastasis in the arch of the first cervical vertebra*



there was no need for instrumented fusion or postoperative immobilisation. Since selective arteriography performed before the operation demonstrated that blood supply to the tumour was limited, the metastasis was not embolised prior to surgery. The histological evaluation was consistent with a metastasis of Hürthle cell carcinoma of the thyroid. MRI of the neck five months later showed no residual tumour mass or recurrence in the neck. The patient did not opt for additional treatment with a targeted agent such as sorafenib.

DISCUSSION

In our patient, rhTSH administration led to symptoms caused by an undetected metastasis in the arch of the first vertebra, secondary to his thyroid carcinoma. His complaints were satisfactorily relieved with NSAID treatment alone. Because of his rapid recovery no glucocorticoids were prescribed and the metastasis was successfully removed by surgery.

In patients with differentiated thyroid carcinoma, rhTSH is frequently administered prior to ^{18}FDG-PET in order to raise circulating TSH levels, thereby improving assessment of the extent of metastases. Although such treatment is generally well tolerated, there are reports of side effects following rhTSH administration. The most common adverse events are nausea (12%) and headache (7%). Post-marketing experience has also shown that rhTSH administration may cause transient flu-like symptoms, including fever, chills, myalgia and headache. Adverse effects of relevance to this case have also been reported. For example, there have been a limited number of reports of patients with known metastases of the central nervous system (CNS) experiencing acute hemiplegia[7] one to three days after rhTSH administration. The package insert for rhTSH mentions that this happened in four out of 55 patients with CNS metastases who were followed in a special treatment protocol.[8] These symptoms have been attributed to local oedema, increased blood flow and/or focal haemorrhage at the site of the cerebral or spinal cord metastases. A case of acute visual loss was reported in a patient with metastasis to the optic nerve.[8] Others have reported sudden, rapid and painful enlargement of locally recurring papillary carcinoma, accompanied by dyspnoea, stridor or dysphonia, which was successfully treated with glucocorticoid therapy.[9,10] A further report concerned a patient who developed haemoptysis and hypoxia three days after rhTSH administration, explained by intratumoural oedema and haemorrhage.[11] Due to the possibility of such side effects, pretreatment with glucocorticoids has been recommended for patients in whom local tumour expansion may compromise vital anatomic structures.[8,9]

In patients with known distant metastases of thyroid carcinoma, doctors should anticipate the possible development of bone or brain metastases. The ^{18}FDG-PET technique is routinely used in our hospital to assess tumour dissemination after stimulation with rhTSH since numerous reports have shown that uptake of the ^{18}FDG-PET tracer is higher in both patients withdrawn from thyroxin to stimulate endogenous TSH and in those stimulated with rhTSH.[1,3,4,12] These findings have been supported by *in vitro* data showing increased uptake of ^{18}F-deoxyglucose in cultured thyroid cancer cells in the presence of TSH.[13] Despite these reports, the current guidelines of the American Thyroid Association (ATA) state that stimulation with endogenous TSH following thyroxin withdrawal or rhTSH may only minimally enhance the sensitivity and specificity of ^{18}FDG-PET scanning.[14]

In summary, we report a patient with sudden onset of neck pain following administration of rhTSH for diagnostic purposes. The symptoms were caused by vertebral metastasis. Patients and clinicians should be aware that rhTSH administration may lead to a sudden increase in blood supply to a tumour or an increase in oedema, thereby causing symptoms due to previously occult metastases of thyroid carcinoma.

ACKNOWLEDGEMENTS

We thank Sally Hill for critically reading the manuscript.

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PHOTO QUIZ

A 76-year-old male with a blue toe and livedo reticularis

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CASE REPORT

A 76-year-old Caucasian male presented with acute kidney injury. His past medical history included hypertension (of unknown duration) and peripheral artery disease, for which percutaneous transluminal angioplasty of the right leg had been performed in 1997. He had smoked 38 pack-years. In the past three days he had experienced a painful right toe. He was not on any oral anticoagulants. On physical examination the patient was lean, not oedematous and his blood pressure was 120/69 mmHg. He had a marked cyanotic right toe (*figure 1*) with absent pedal pulses and livedo reticularis most prominently on the left knee (*figure 2*). Laboratory investigation showed an erythrocyte sedimentation rate of 44 mm/hour, mild leucocytosis of 10.9 x 10^9/l with an eosinophil count of 0.58 x 10^9/l. Serum creatinine was 213 µmol/l (baseline creatinine 97 µmol/l) with an estimated glomerular filtration rate of 26 ml/min. Urinanalysis revealed 2.12 g/l of protein and no erythrocyturia. Antineutrophil cytoplasmic antibodies were negative. Ultrasound demonstrated relatively small kidneys of 9.1 cm and 9.5 cm and an infrarenal abdominal aneurysm. Computer tomography (CT) imaging showed no renal artery stenosis and confirmed the aneurysm, which measured 5.6 cm and contained atherosclerotic plaques and a mural thrombus.

Figure 1. *Cyanotic digit 3 of right foot*

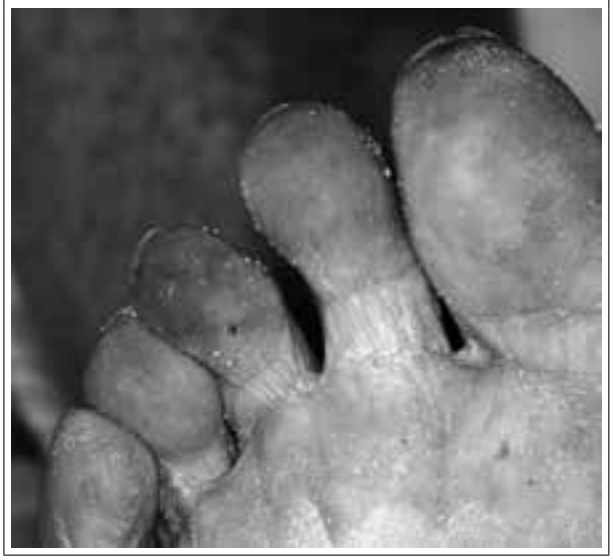
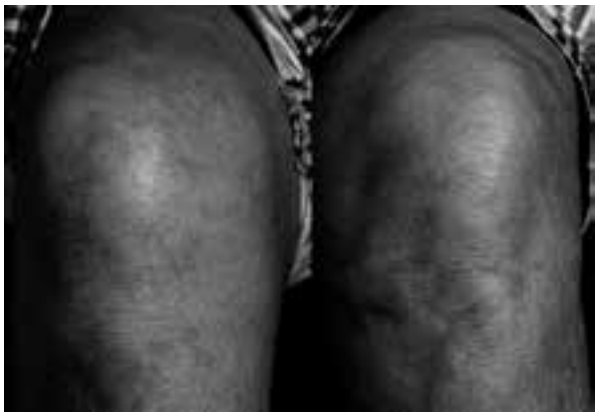


Figure 2. *Livedo reticularis most prominently on left knee*



WHAT IS YOUR DIAGNOSIS?

See page 261 for the answer to this photo quiz.

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PHOTO QUIZ

A breathtaking response to tuberculosis therapy

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A 31-year-old Malaysian woman, diagnosed with tuberculosis (TB) of the mediastinal lymph nodes, presented to our outpatient clinic because of progressive dyspnoea and malaise three weeks after initiation of anti-TB therapy. The patient reported severe dyspnoea, orthopnoea and high fever. At that moment she was taking rifampicin, isoniazid, pyrazinamide and ethambutol. Before treatment she had complained of fever, weight loss and night sweating, but no dyspnoea was reported.

On physical examination she had a blood pressure of 121/74 mmHg with a tachycardia of 120 beats/minute. Her temperature was 40.1 °C and she had a breathing frequency of 30/minute with an arterial oxygen saturation of 99% without supplemental oxygen. Laboratory findings revealed a mild leucocytosis (11.3 x 10^9/l) and a C-reactive protein of 248 mg/l. Electrocardiogram showed a sinus tachycardia with nonspecific T-wave abnormalities.
The chest X-ray after three weeks of therapy is shown in *figure 1,* together with the chest X-ray before the start of therapy.

WHAT IS YOUR DIAGNOSIS?

See page 262 for the answer to this photo quiz.

Figure 1. *Chest X-ray before (A) and after (B) three weeks of anti-TB treatment*

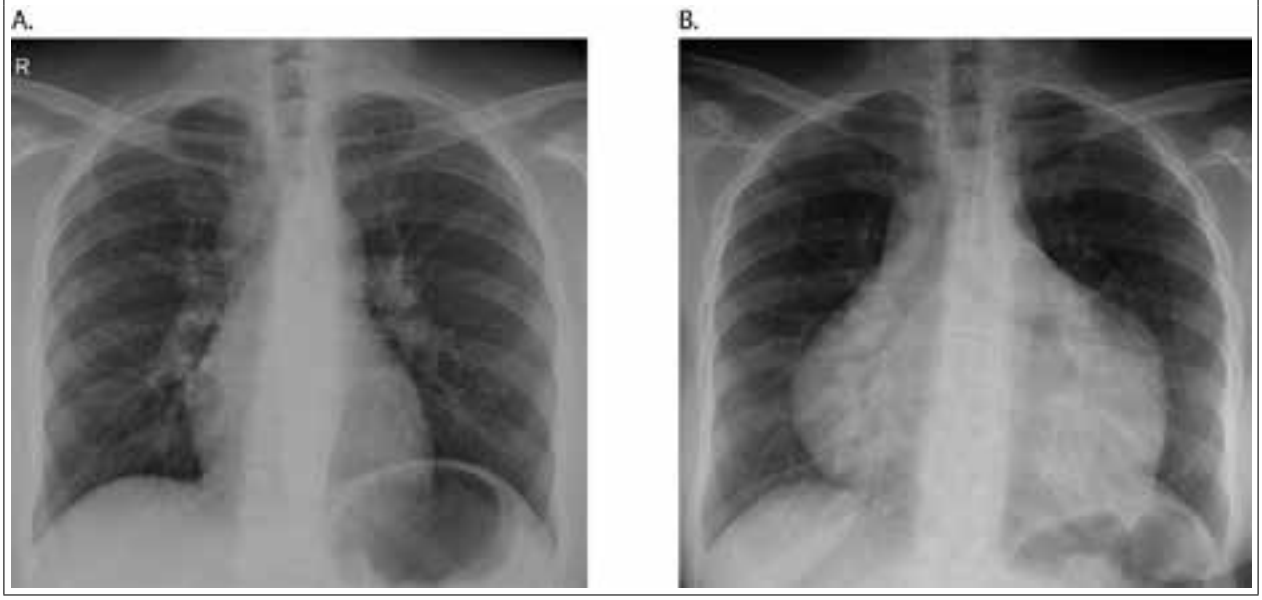

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PHOTO QUIZ

Chronic blepharitis

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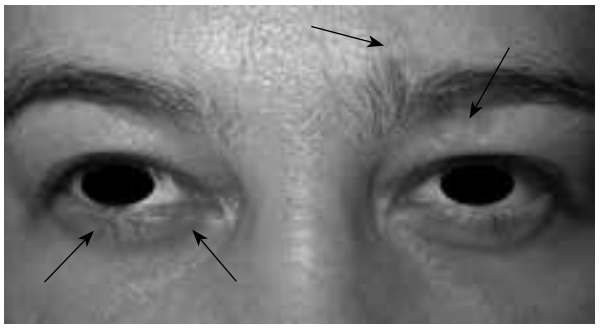
CASE REPORT

A 35-year-old woman sought medical advice for an asymptomatic lesion of the lower right eyelid, which had been present for one year and was refractory to topical antifungals and antibiotics, and dermocorticosteroids. The lesion involved the whole eyelid, was erythematous, oedematous, and atrophic at its inner part (*figure 1*). She also reported other cutaneous lesions including one erythematous lesion with an atrophic centre on the upper left eyelid, and one smooth erythematous plaque above the left eyebrow, which had developed three months previously *(figure 1)*. Physical examination was otherwise unremarkable. Routine blood examination including blood cell count, serum creatinine, urinalysis, and liver function tests were normal or negative.

Figure 1. *Erythematous oedema of the whole lower right eyelid with atrophy at the inner part, an annular lesion with an atrophic centre below the left eyebrow related to discoid lupus erythematosus, and erythematous oedematous plaque above the left eyebrow due to lupus erythematosus tumidus*



WHAT IS YOUR DIAGNOSIS?

See page 263 for the answer to this photo quiz.

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PHOTO QUIZ

A fatal rash

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CASE REPORT

A 45-year-old south-Indian male presented with a new-onset 'rash' and 'skin tightness' that initially appeared on his face and progressed to involve his neck, trunk and extremities over a period of two months. He was referred to our institution with a presumed diagnosis of cutaneous T-cell lymphoma after skin biopsy. On physical examination, there was generalised lymphadenopathy, erythematous scaly papules, plaques and nodulo-tumoral lesions over the face, neck, trunk and bilateral extremities (*figure 1*).

The haemogram revealed leucocytosis (29,400/mm$^{3)}$ and the peripheral smear showed 28% atypical cells with convoluted/ indented nuclei. Biochemical panel showed hypercalcaemia (19 mg/dl (normal range: 8.4-10.2)), abnormal renal function (serum creatinine: 1.8 mg/dl (normal range: 0.8-1.5)) and elevated lactate dehydrogenase (LDH) [1154 IU/l (normal range: 300-600)]. Skin biopsy revealed dermal lymphoid infiltrates with extensive epidermotropism and epidermal destruction (*figure 2*). On immunohistochemistry, tumour cells showed positivity for CD3, CD5 and CD25, loss of CD7 and scattered large cells expressed CD30.

Figure 1. *Diffuse erythematous scaly papules and plaques over the neck, upper extremities and trunk and nodulo-tumoral lesions over the face*

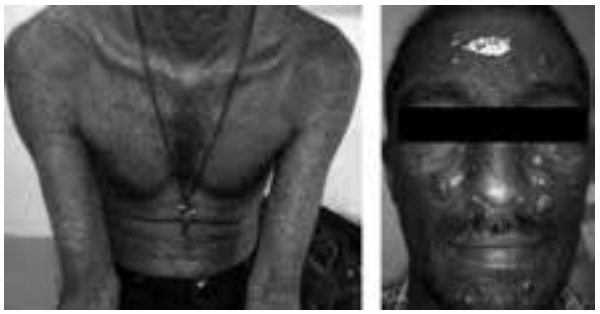
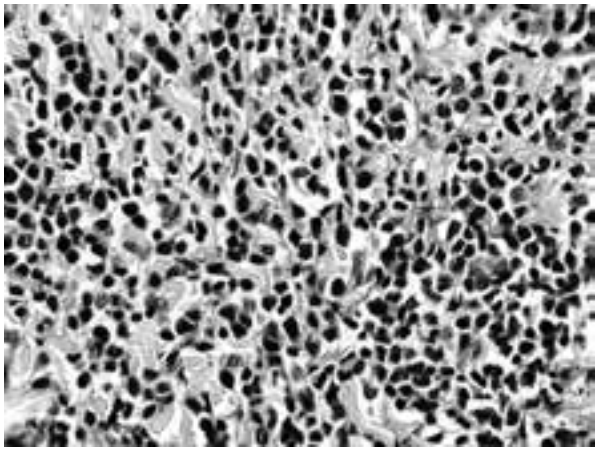


Figure 2. *H & E (x400): Section from skin, dermis shows diffuse infiltration by atypical lymphoid cells with moderate cytoplasm and convoluted nuclei*



WHAT IS YOUR DIAGNOSIS?

See page 264 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 257)

A 76-YEAR-OLD MALE WITH A BLUE TOE AND LIVEDO RETICULARIS

DIAGNOSIS

Based on the dermatological features (blue toe and livedo reticularis) and eosinophilia in addition to acute kidney failure, the diagnosis of spontaneous cholesterol crystal emboli (CCE) was made.[1]

CCE is iatrogenic in the majority of cases, with angioplasty (50%), vascular surgery (15%) and long-term anticoagulant therapy (76%) being the most common aetiological factors.[2] Spontaneous CCE are rare, with an estimated incidence of only 1.9%.[3] Typically, a patient with CCE is a lean, smoking male suffering from other manifestations of atherosclerosis.[1] Almost all patients with CCE (97%) have plaques in their thoracic aorta and 67% of patients have an aortic abdominal aneurysm.[2] Cholesterol emboli cause arterial occlusion leading to end-organ damage, involving most commonly the brain, kidneys, gastrointestinal tract, the skin and skeletal muscles of the lower extremities. Classic clinical features are livedo reticularis and cyanotic toes. Histopathology is regarded as the golden standard.[4] However, because on the one hand the combined clinical scenario and features in addition to the laboratory findings were very suggestive of the diagnosis of CCE and on the other hand performing a biopsy had no clear therapeutic consequences, a confirmatory kidney biopsy was not performed in our patient. Also, biopsy of the affected skin or skeletal muscles is performed infrequently because it may lead to poor healing at the sampling site.[1]

To date there is no specific therapy for CCE. Because this disorder is a manifestation of atherosclerosis, modification of traditional risk factors for atherosclerosis such as smoking, hypertension and serum cholesterol is strongly advised.[1] Angiotensin-converting enzyme inhibitors or direct angiotensin receptor blockers and antiplatelet therapy could be considered. Since anticoagulant therapy may aggravate CCE, these drugs are contraindicated.[1] Surgical therapy has been shown to be effective in decreasing the rate of future embolism. The role of corticosteroids in CCE is still inconclusive.

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ANSWER TO PHOTO QUIZ (PAGE 258)

A BREATHTAKING RESPONSE TO TUBERCULOSIS THERAPY

TUBERCULOUS PERICARDITIS

In this case the pericardial effusion which started three weeks after initiation of anti-TB treatment was highly suggestive of tuberculous pericarditis, as a sign of a paradoxical reaction. Cardiac ultrasound confirmed the presence of pericardial effusion and signs of inflow obstruction. Pericardial drainage was performed and 700 ml of serous fluid was drained. Polymerase chain reaction of the pericardial fluid was positive for mycobacterium tuberculosis. We continued the anti-TB regimen, and started prednisolone treatment in a dosage of 60 mg a day according to current guidelines.[1]

Paradoxical reactions during TB treatment are thought to occur in 20-25% of the patients.[2,3] Although no strict definition of a paradoxical reaction exists, an often used definition is: clinical or radiological worsening of pre-existing tuberculous lesions or the development of new lesions not attributable to the normal course of disease in a patient who initially improves on anti-tuberculosis therapy and in whom the onset of the paradoxical response is at least two weeks after initiation of this treatment.[4] The most common sites involved in paradoxical reactions are the central nervous and respiratory system.[4] The pathophysiology of paradoxical responses in TB, as well as the subsequent restoration of the skin test,[5] is not well known. Suggested mechanisms include: 1) reversal of immunosuppression due to the TB itself, 2) release of large amounts of endotoxins from destroyed bacilli and 3) improvements in the nutritional status.[5] The therapeutic approach to paradoxical reactions has not been well studied; guidelines state that that prednisone or methylprednisolone should be started at a dose of about 1 mg/kg.[1] In our patient this meant that the dosage of 60 mg she was receiving because of the pericardial involvement was sufficient against a paradoxical reaction as well. Control cardiac ultrasound one week later showed no progression of the pericardial effusion. After three weeks she had no complaints of orthopnoea or dyspnoea on exertion.

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ANSWER TO PHOTO QUIZ (PAGE 259)

CHRONIC BLEPHARITIS

DIAGNOSIS

The diagnosis of lupus blepharitis was suggested clinically because of the typical skin lesions of discoid lupus erythematosus (LE) involving the upper left eyelid and of LE tumidus of the forehead, both confirmed by cutaneous biopsy. The search for antinuclear, anti-DNA, anti-extractable nuclear antigen antibodies was negative. Topical tacrolimus was ineffective, but hydroxychloroquine resulted in a dramatic improvement in all the lesions within three months.

Blepharitis is an inflammatory condition of the eyelid margin, anatomically subdivided into posterior and anterior variants. Posterior blepharitis is related to dysfunction of the meibomian glands.[1] Common causes of anterior blepharitis, involving the lashes and associated oil glands, are infections and inflammatory conditions (e.g. rosacea, psoriasis, atopic dermatitis).[1]

Chronic cutaneous LE is subdivided into different entities including discoid LE and LE tumidus mainly on sun-exposed areas, which may be associated with or develop organ and system involvement.[2] Discoid LE is the most common form of chronic cutaneous LE, characterised by erythematous, scaly, atrophic or oedematous lesions located primarily in sun-exposed areas, including the scalp, face, ears, and arms. Skin biopsy shows an hyperkeratotic or atrophic epidermis depending on the stage of the disease, vacuolar alteration of the basal layer, and a superficial and deep, perivascular and periadnexal lymphocytic infiltrate. Involvement of the eyelid with a predilection for the external and inferior portions of the eyelid has been reported; diagnosis is often delayed.[3,4] LE tumidus mainly affects sun-exposed sites and is characterised by non-scarring, erythematous, swollen, urticaria-like bumps and plaques with histologically no surface changes, but dermal infiltrate in a perivascular and periadnexal distribution and abundant interstitial mucin deposition. Lupus blepharitis should therefore be considered as a possible diagnosis in chronic blepharitis refractory to medical management and eyelid hygiene. The diagnosis requires biopsy then and treatment consists of topical corticosteroids or tacrolimus, and antimalarial drugs associated with photoprotection. Early diagnosis and appropriate treatment should prevent complications such as permanent scarring, disorganisation of the mucocutaneous junction, and symblepharon formation.[4]

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ANSWER TO PHOTO QUIZ (PAGE 260)

A FATAL RASH

The diagnosis of adult T-cell leukaemia/ lymphoma (ATL) was confirmed by serological analysis demonstrating antibodies to human T-lymphotropic virus 1 (HTLV-I). The patient was started on combination chemotherapy but eventually died from progressive disease with fatal pulmonary haemorrhage.

ATL is a peripheral mature T cell neoplasm caused by the retrovirus HTLV-I. HTLV-I is transmitted vertically, sexually or parenterally. The epidemiology of ATL corresponds to HTLV-I endemic zones such as southwest Japan, the Caribbean basin, north-east Iran, Central and South America and Africa. There have been only few reports from the Indian subcontinent. Close differential diagnoses include cutaneous T cell lymphoma, anaplastic large cell lymphoma and T-prolymphocytic leukaemia.[1,2]

ATL is subdivided according to the Shimoyama classification into four major clinical variants: acute (leukaemic) and lymphomatous (aggressive ATL) and the chronic and smouldering (indolent ATL). The acute variant accounts for up to 60% of cases and has a poor prognosis with median survival from six months to a year. It usually presents with generalised lymphadenopathy, hepatosplenomegaly, bone marrow involvement, hypercalcaemia, lytic bone lesions and skin manifestations.[1]

The 'flower' or 'clover-leaf' cells in the peripheral smear are considered pathognomonic. Tumour cells are positive for T cell markers such as CD2, CD4, CD5, CD45RO, CD29, T cell receptor αβ and usually lack CD7, CD8 and have reduced CD3 expression. CD25 and CD52 are expressed in most cases. Poor prognostic markers include poor performance status, age >40 years, hepatosplenomegaly, more than three involved lesions, elevated LDH and hypercalcaemia. The type of skin eruption is an independent prognostic marker and the worst prognosis is associated with the erythrodermic followed by nodulo-tumoral and multipapular eruptions.[1,3]

Aggressive ATL presents a therapeutic challenge due to intrinsic chemoresistance, large tumour burden and severe immunocompromise. Treatment options include chemotherapy, antiviral regimens, haematopoietic stem cell transplantation and investigational clinical trials. However, most patients with aggressive ATL relapse despite current day therapy.[4]

In conclusion: a fatal presentation of acute adult T-cell leukaemia/lymphoma posing a diagnostic challenge in a non-endemic region.

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SPECIAL REPORT

Chest pain in sickle cell disease

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ABSTRACT

The differential diagnosis of chest pain in a patient with sickle cell disease is difficult and may encompass several serious conditions, including chest syndrome, pulmonary embolism and infectious complications. In this manuscript we provide an overview on the various underlying diseases that may cause chest pain in patients with sickle cell disease and provide clues for a proper diagnostic workup.

KEYWORDS

Acute chest pain, myocardial infarction, sickle cell disease, vaso-occlusive crisis.

INTRODUCTION

A clinical pathology conference is held each trimester at the Department of Internal Medicine of the Academic Medical Centre Amsterdam. A few weeks before the conference, a senior resident is presented with a 'paper' case to be solved. The resident is provided with some but not all details on the case, such as clinical, laboratory and radiological data (no reports). Based on this information, the resident puts together a case with a focus on clinical reasoning leading to a provisional diagnosis. Afterwards, the clinician who provided the case reveals the actual diagnosis and clinical course. Here, a recent case is reported of a young woman with sickle cell anaemia who presented with acute chest pain.

THE CASE

A 35-year-old woman presented at the emergency room with chest pain. She was known with sickle cell anaemia combined with heterozygous alpha-thalassemia (-α/αα) with frequent painful crises. The sickle cell anaemia was complicated by symptomatic cholecystolithiasis and avascular necrosis of the femoral heads, for which cholecystectomy and bilateral total hip replacement had been performed. Two years before presentation she had had an ischaemic stroke from which she had residual impairment. Due to multiple blood transfusions she had developed iron overload for which she was treated with the iron chelator deferiprone. Because of chronic pain she frequently used cannabis, methadone, NSAIDs and paracetamol. As a consequence, managing her painful crises had become increasingly difficult. A port-a-cath had been placed to secure venous access. Recently vitamin D deficiency with severe hypocalcaemia was diagnosed, for which she was prescribed colecalciferol and calcium carbonate.

At presentation she complained of chest pain located around the sternum and the port-a-cath since a few days. The pain worsened upon movement; otherwise no provoking factors could be discerned. The patient had not been ill, nor had she had any fever. She was not dyspnoeic and she had no cough. She had suffered palpitations. In addition, she had experienced painful muscle cramps. On physical examination the patient appeared in pain but not ill. Her blood pressure was 145/55 mmHg and her pulse 90 beats/minute and regular; the temperature was normal. Palpation of the skin overlying the port-a-cath was painful, but there was no redness or swelling. Otherwise the physical examination was unremarkable.

CLINICAL REASONING

This case concerns a young woman with severe and complicated sickle cell anaemia presenting with (semi) acute chest pain, palpitations and muscle cramping.

Sickle cell disease (SCD) is characterised by recurrent painful vaso-occlusive crises and progressive organ damage leading to premature death.

A single mutation in the β-globin gene results in the formation of haemoglobin S (HbS). SCD is caused by homozygosity for this mutation (sickle cell anaemia; HbSS), or by heterozygosity for the HbS gene in combination with other haemoglobinopathies such as HbSC or HbS-β-thalassaemia. Upon deoxygenation, a hydrophobic motif in the HbS tetramer causes the HbS molecules to polymerise resulting in sickling of the erythrocytes.[1] Sickled erythrocytes interact with neutrophils and endothelium which leads to vaso-occlusion resulting in ischaemia and haemolysis and subsequently activation of inflammation and coagulation. Next, reperfusion of ischaemic tissues causes oxidative stress (reviewed by Nur, *et al.*[2]). In addition to the acute ischaemic injury to affected organs by vaso-occlusion, these processes lead to vasculopathy and chronic organ dysfunction such as cerebrovascular disease, renal failure and pulmonary hypertension.[3]
Alpha thalassaemia results from impaired production of one to three of four alpha globin chains. Alpha thalassaemia minima (-α/αα) is generally asymptomatic, but in patients with SCD, concurrent alpha thalassaemia increases the frequency of vaso-occlusive crises, acute chest syndrome and osteonecrosis.[4,5]

This patient presents with three symptoms which may or may not be related: (semi) acute chest pain, palpitations and muscle cramping. As (semi) acute chest pain may indicate serious pathology, this symptom should be explored with priority.

Painful vaso-occlusive crisis
Periodic episodes of excruciating musculoskeletal pain are the hallmark of SCD. Although the majority of crises arise spontaneously, they can be provoked by external factors such as infections, stress, dehydration or extreme physical exertion. The main determinants for the frequency of crises are the haematocrit and percentage HbF.[6] Recurrent vaso-occlusive crises give rise to progressive organ damage such as avascular necrosis of the femoral heads, renal failure, neurological impairment and functional asplenia.
Painful crises are the most common reason for admission and a major cause of morbidity and reduced quality of life.[7] In adult patients, frequent pain is even associated with a higher mortality rate. The variability of episodes requiring clinical care is high; up to 40% of patients have less than one crisis per year, whereas 1% of patients have more than six episodes.[6] Of note, a recent prospective cohort study using daily self-assessment diaries revealed that pain is even more prevalent; pain was reported in 55% of analysed patient days, while 29% of patients reported pain almost daily. In addition, the majority of crises were managed at home.[8] The discrepancy between the actual prevalence of pain and incidence data derived from studies defining painful crisis by the need for utilisation of health care may lead to miscommunication and undertreatment. For example, a survey among haematologists and emergency department physicians regarding their perceptions concerning sickle cell pain revealed that a considerable portion of them believed that >20% of their patients are addicted to opioids.[9]
This patient is known with chronic pain, analgesia use and frequent crises; however, she did not report to have a crisis at presentation. Therefore a vaso-occlusive crisis as the cause of the present chest pain is less likely.

Acute chest syndrome
Acute chest syndrome is the second most common cause of hospitalisation in SCD. It is a serious clinical condition and a major cause of death in SCD. The aetiology is multifactorial; the syndrome is set off by pulmonary infection or infarction by bone marrow-derived fat emboli. Atelectasis resulting from hypoventilation due to musculoskeletal pain and increased airway reactivity may further add to the pathophysiology. Local shunting leads to deoxygenation and sickling of erythrocytes and vaso-occlusion (reviewed by Gladwin, *et al.*[10]). Clinically, the acute chest syndrome is defined by the presence of a new pulmonary infiltrate involving at least one lung segment in combination with fever (80%) and pulmonary symptoms such as coughing (62%), dyspnoea and/or chest pain (40%). Typically, the acute chest syndrome presents three days after the start of a vaso-occlusive crisis. Rapid diagnosis and treatment are warranted; around 13% of patients require ventilation during the course of the disease and mortality is 3%.[10] Although the index of suspicion for this syndrome should always be high, this diagnosis seems less likely based on the absence of pulmonary symptoms and fever in this patient.

Causes indirectly related to SCD
The life expectancy of patients with sickle cell anaemia is greatly diminished at approximately 40 years.[11-13] Death is frequently sudden and unexpected (40.8%) and commonly occurs within 24 hours after presentation to the hospital.[14] Cardiopulmonary causes account for a significant proportion of deaths. In one single-centre cohort study in 240 patients with SCD, 43 patients died during a five-year observation period (median age 39 years). Of these, 11 died of cardiac causes (pulseless arrest (n=5), congestive heart failure (n=3), myocardial infarction (n=3)) and six of pulmonary causes (including four patients with fatal pulmonary embolism).[13] Therefore, causes of chest pain unrelated to SCD should be seriously considerated.

Pulmonary embolism
SCD is characterised by chronic activation of coagulation; nearly all components of haemostasis are altered

(reviewed by Ataga, *et al.*[15]). The actual risk of venous thromboembolism (VTE) attributable to SCD is not well known, largely because of difficulties in distinguishing it from fat emboli or thrombosis in situ, but also because of the high prevalence of (SCD-related) comorbidities in patients with SCD. Furthermore, almost all markers of coagulation including D-dimers are disturbed in SCD,[16] which hampers the diagnostic process. However, studies indicate that SCD is probably an independent risk factor for VTE. A retrospective analysis of the National Hospital Discharge Survey (US) revealed that 0.44% (7000 of 1,581,000) of patients with SCD had a discharge diagnosis of PE compared with 0.12% (59,000 of 48,611,000) of African Americans without SCD. The incidence of deep vein thrombosis did not differ, indicating that at least some of the cases of PE may have been in situ thrombi.[17] In a recent case-control study the attributable risk of sickle cell trait (SCT) for VTE was calculated to be 7%; the odds ratio (OR) for VTE was 1.8 (95% CI, 1.2-2.9) in subjects with SCT versus subjects homozygous for the wild-type allele. The OR for pulmonary embolism and sickle cell trait was even higher at 3.9 (CI 2.2-6.9).[18]
Although our patient does not have 'typical symptoms' of pulmonary embolism, this diagnosis should not be rejected before a satisfactory alternative diagnosis is made.

Cardiac causes
Cardiac complications are a leading cause of morbidity and mortality in adult patients with SCD. Cardiac abnormalities can be divided into three major groups. First, cardiac output is increased due to chronic anaemia and recurrent episodes of hypoxia. In addition, pulmonary hypertension is found in up to 6% of patients with SCD (with chronic haemolytic anaemia as central risk factor).[19] Together, this may result in progressive diastolic and systolic dysfunction and eventually in overt left- as well as right-sided chronic heart failure (reviewed by Voskaridou, *et al.*[20]). Secondly, patients with SCD are prone to arrhythmias; a prolonged QTc time is found in up to 40% of patients in steady state.[21] The use of medication such as methadone contributes to QTc prolongation. Furthermore, continuous monitoring of 30 patients during the first 24 hours of a vaso-occlusive crisis revealed arrhythmias in 24 patients, of both atrial (60%) and ventricular (67%) origin. Nine of these patients even had 'complex arrhythmias' including two with episodes of ventricular tachycardia.[22] Thirdly, patients with SCD have an increased risk of ischaemic heart disease. Acute myocardial infarction is a common cause of sudden death in SCD.[13,23] However, cardiac ischaemia is often insufficiently recognised as a typical vaso-occlusive crisis presents with diffuse musculoskeletal pain or atypical chest pain and patients are young. Therefore, the true incidence of cardiac ischaemia is not well known.

Our patient has new palpitations and atypical chest pain. Considering the possible sequelae, cardiac pathology should be excluded.

Other causes
As we have only sparse knowledge about the symptoms and signs in this patient, at this point many alternative explanations for the chest pain (including malfunction or infection of the port-a-cath, pleuritic, pericardial, myogenic, osteogenic (osteomalacia), costochondral and gastrointestinal causes) cannot be definitely ruled out.

CLINICAL DIAGNOSIS

Based on the sparse clinical information provided, the most obvious causes of chest pain in a patient with SCD, namely vaso-occlusive crisis and acute chest syndrome, seem less likely.
Considering the high incidence of life-threatening cardiac and thromboembolic events in patients with SCD, first of all myocardial ischaemia should be ruled out and next pulmonary embolism.

ADDITIONAL TESTING

Guided by the differential diagnosis, additional laboratory testing and an electrocardiogram were performed (*table 1* and *figure 1*). Laboratory results showed anaemia with

Table 1. *Laboratory results*

Variable	Reference range		
Haematology			
Haemoglobin (mmol/l)	7.4-9.9	5.2	↓
Leucocyte count (x 10^9/l)	4.5-11.0	17.2	↑
Platelet count (x 10^9/l)	150-400	334	
Chemistry			
Calcium (mmol/l)	2.20-2.60	1.42	↓
Phosphate (mmol/l)	0.70-1.50	0.77	
Creatinine (µmol/l)	50-90	52	
Albumin (g/l)	35-50	44	
Alkaline phosphatase (U/l)	40-120	158	↑
γ-Glutamyltransferase (U/l)	5-40	22	
ASAT (U/l)	10-30	36	↑
ALAT (U/l)	5-35	25	
Lactate dehydrogenase (U/l)	75-250	481	↑
Bilirubin (total, µmol/l)	0-17	26	↑
Troponin-I (µg/l)	0.00-0.04	1.18	↑
Creatine kinase-MB (µg/l)	0.0-7.0	1.7	
Endocrinology			
25 (OH) vitamin D (nmol/l)	50-250	5	↓
Parathyroid hormone (pmol/l)	1.5-7.6	84.3	↑

Figure 1A. *Electrocardiogram at presentation (calcium = 1.42 mmol/l)*

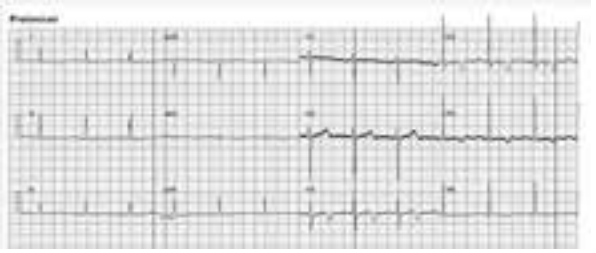
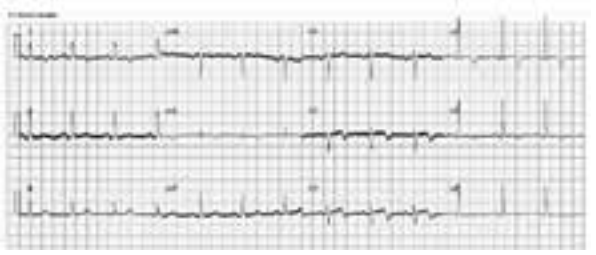


Figure 1B. *Electrocardiogram after calcium suppletion (calcium = 2.27 mmol/l)*



increased total bilirubin, lactate dehydrogenase levels and decreased haptoglobin levels compatible with chronic haemolysis. The leucocyte count was increased. The renal function was normal. The calcium level was very low at 1.42 mmol/l, while the albumin level was normal. Also the magnesium level was slightly decreased, but levels of the other electrolytes, including phosphate, were normal. The vitamin D level was low, whereas the parathyroid hormone level was high. Lastly, the marker of cardiac muscle tissue injury troponin-I was increased.

Electrocardiography showed a sinus rhythm of 74 beats/minute, an intermediate axis, normal PR and QRS conduction intervals, but a prolonged QTc time (525 msec). Furthermore, terminal negative T waves were observed in V3-V6, I and aVL (*figure 1*, upper panel); these abnormalities persisted after correction of serum calcium and nitrate administration (*figure 1*, lower panel). She did have relief of the chest pain upon nitrate administration. Echocardiography showed a non-dilated left ventricle with slight hypokinesia apical anterior (in agreement with the suspected area of ischaemia), but otherwise normal systolic and diastolic function. Right ventricular function was normal. During prolonged monitoring cardiac arrhythmias were not observed.

DIAGNOSIS

Chest pain due to myocardial ischaemia.

DISCUSSION

Considering the high oxygen extraction in the heart muscle and the fact that hypoxia promotes sickling of erythrocytes, the heart seems to be relatively protected from ischaemic damage in SCD. Yet, myocardial infarction is frequently found at autopsies.[11,23] Strikingly, atherosclerotic changes are usually absent. In one autopsy series, seven of 72 consecutive patients with SCD had myocardial infarction, but overt obstructive lesions were absent in all of them.[23] Cardiac ischaemia in SCD is rather caused by pathology at the level of the microvasculature and has a multifactorial aetiology. As in other vaso-occlusive crises, the process may be initiated by increased adherence of sickled erythrocytes to activated endothelium. Abnormally activated platelets and leucocytes sequestrate in the obstructed vessels and release inflammatory mediators. Haemolysis leads to the release of free haemoglobin which acts as an NO scavenger. Rheological changes due to hyperviscosity and structural changes of the microvasculature further add to the pathophysiology (reviewed by Voskaridou, et al.[20]). The recognition of cardiac ischaemia is hampered by the fact that chest pain in patients with SCD is often attributed to other causes. Furthermore additional diagnostic tests may not be discriminating either. Non-specific ST-T wave changes and signs of abnormal repolarisation are common electrocardiographic findings in SCD. Frequent intramuscular injection of analgesics may result in a rise in muscle enzymes which interferes with the interpretation with respect to myocyte damage. Lastly, in the absence of atherosclerotic lesions, coronary angiogram is typically normal.

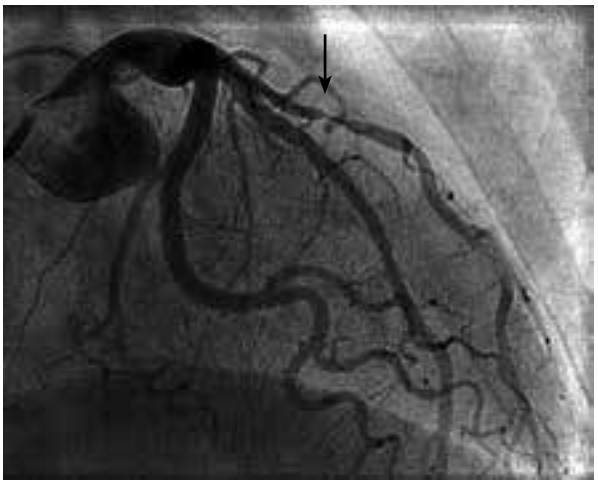
The muscle cramping is likely due to severe hypocalcaemia. Both calcium and high doses of colecalciferol were prescribed previously; however given the very low vitamin D level incompliance was suspected. The compensatory increased parathyroid hormone level supports the diagnosis of primary vitamin D deficiency.

The palpitations may indicate the presence of paroxysmal tachyarrythmias although not formally registered. Possible causes of tachyarrhythmias in this patient include anaemia, myocardial ischaemia, hypocalcaemia and (though less likely) torsade de pointes as complication of the prolonged QTc time (promoted by hypocalcaemia and methadone use).

EPILOGUE

Coronary angiography showed a subtotal stenosis (80-90%) in D1; the other coronary arteries appeared patent (*figure 2*). Although the pathophysiologies of atherosclerosis and vasculopathy of SCD have many overlapping features, previous cases of myocardial

Figure 2. *Coronary angiogram shows a subtotal stenosis in D1 (arrow), whereas the other arteries appear unaffected.*



infarction due to an atherosclerotic lesion have not been reported in patients with SCD. An important distinction between atherosclerosis and vasculopathy of SCD is the absence of atheromas in the latter, probably due to very low total cholesterol and LDL levels in patients with SCD.[24] Besides smoking cannabis, the patient had no risk factors for cardiovascular disease; however, evidence exists that low 25(OH) vitamin D levels are associated with an increased risk of cardiovascular disease.[25] Alternative causes for an isolated coronary stenosis, e.g. a congenital stenosis or muscular bridging, were unlikely based on the coronary angiography.

The patient was referred for percutaneous coronary intervention. A drug-eluting stent was placed via the arteria femoralis dextra. After this procedure the chest pain was relieved.

A thorough history revealed that the patient had stopped taking all the prescribed medication except for methadone many weeks ago. This explains why the serum calcium levels were very low despite high doses of colecalciferol and calcium.

Patients with SCD are vulnerable due to often extensive comorbidity and their increased risk for some common life-threatening conditions. This case illustrates the urgency to avoid preconceived opinions and the need to look beyond 'the usual suspects' in such patients presenting with acute pain.

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SPECIAL REPORT

The challenge of multidisciplinary research: improving diabetic pregnancy together

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ABSTRACT

Improving diabetic pregnancy outcome is a goal shared by many involved specialists. Despite proper glucose control, the incidence of maternal and perinatal complications is very high, including a high risk for pre-eclampsia, congenital malformations, perinatal mortality and macrosomia. To improve outcome, not only collaborating in the doctor's office is required but also participation in critical evaluation of our treatment strategies by means of randomised clinical trials.

KEYWORDS

Diabetes, pregnancy, multidisciplinary research

PROBLEM

Modern health care is at a turning point. Over the last decades increasing insights into physiology and pathophysiology of disease, as well as a raising number of technical and pharmaceutical tools to interfere with the natural course of disease, have enriched diagnostic and therapeutic opportunities. Until recently, these new opportunities were welcomed and introduced with limited concerns on costs and effectiveness.

However, recent social and economic developments have changed the context. Budget pressure forces politicians to make choices between the reimbursement of different medical interventions. The Minister of Health recently asked doctors and patients to advise in these difficult choices. Such choices can only be made in view of knowledge of the effectiveness of these interventions. Possibly, the reimbursement status of Continuous Glucose Monitoring (CGM) during pregnancy will be reconsidered since solid scientific support is lacking.

DIABETES AND PREGNANCY

A decade ago, a nationwide prospective cohort study was performed on pregnancy outcome of women with DM type 1 (n=323). During pregnancy, the mean HbA_{1c} value was reassuring, namely 6.2% (44 mmol/mol). However, the incidence of maternal and perinatal complications was remarkable. Most outstanding were the relative risks for pre-eclampsia (12.1), congenital malformation (3.4), perinatal mortality (3.5) and macrosomia (4.5).[1] Two important conclusions were made. First, women with diabetes still have high-risk pregnancies and second, near-normal HbA_{1c} values do not automatically translate into good pregnancy outcome.

Consequently, the question arose whether or not we have reached the maximum effect on pregnancy outcome that strict glycaemic control can accomplish. Can pregnancy outcome be further improved by additional interventions focusing on glycaemic control or do we need to target other features?

CGM provides detailed information on daily glycaemic profiles and may provide an opportunity to further improve pregnancy outcome of women with diabetes. 'Does the additional use of CGM improve pregnancy outcome?' Gynaecologists and endocrinologists together wrote a research proposal to address this matter in the GlucoMOMS trial.[2] After ZonMW funding was obtained, indicating the relevance, preparations for a national randomised clinical trial (RCT) started.

Meanwhile, the Health Care Insurance Board (CVZ) discussed reimbursement of this promising new tool. An invited review of literature was written in which it was clearly stated that evaluation of CGMS during pregnancy was lacking.[3] Furthermore, the Endocrine Society Task Force formulated practice guidelines to identify patient groups that are most likely to benefit from CGM, in which pregnancy was not mentioned.[4,5] Nevertheless, CGM came to be reimbursed for use during diabetic pregnancy. It

should be noted that reimbursement facilitates evaluation, but does not imply that doctors have to prescribe CGMS to their patients. In contrast, an often heard critique is that insurance companies take over control from doctors on the content of care. The fact that CGM is currently reimbursed allows its evaluation. Without such evaluation, it might very well be that the reimbursement status will be reconsidered in the coming years.

REAL TIME VERSUS OFFLINE

CGM can be applied in two fundamentally different ways. First, as a retrospective instrument that stores a large number of glucose measurements per day. The patient is blinded for the measurements, and data from the CGM together with a detailed diary can be analysed retrospectively. Patients can be educated on the basis of the daily graphs and insulin treatment can be adjusted. The real time (RT) CGM is a more extensive strategy. Glucose measurements are directly communicated to the patient by displaying the real time glucose values. Furthermore, trends in glucose levels are shown and alarms can be set for upper and lower limits. Patients can upload their data onto their computer and detailed reports are provided in graphs, charts and tables. Adjustments in insulin dose can be made directly. Both techniques require blood glucose meter readings (fingersticks) at least 3-4 times a day to recalibrate and verify the glucose sensor.

The complex strategy of RT-CGM requires a highly motivated patient. Due to a major role of this 'human factor', RT-CGM is not appropriate for every patient. The correct prerequisites for pregnant women to be eligible for RT-CGM use have yet to be clarified. It remains unclear which group of patients would benefit from the use of CGM during pregnancy, either retrospective or real time. Doctors will continue to be confronted with the question of which type of monitor to use, if any, for which patient, at least until research provides clarity. Initiatives such as the GlucoMOMS trial are of great importance for an evidence-based foundation of CGM use in the future.

CONSORTIUM

The Dutch Obstetric Consortium is a national research network that has been conducting multicentre RCTs since 2003. Over 80 hospitals contribute to multicentre RCTs facilitated by the consortium.[6] The consortium on women's health research is not a formal structure, but rather an informal agreement between different researchers who all want to execute multicentre studies on comparative effectiveness and health care efficiency research. Each project contributes financially to a common infrastructure. The central office of the consortium is located at the AMC in Amsterdam, and is responsible for central and local approval of the trials by the medical ethics commissions. Once the financial and administrative issues are covered, a trial is initiated throughout the country. Each hospital is part of a cluster, associated around a perinatal centre (mostly academic hospitals). The logistics of the trials are covered by the practical task forces: the research nurses, guided by cluster coordinators (gynaecologists). They manage daily local logistics, inform the hospitals on the ongoing trials, handle patient counselling, and perform data entry. This streamlined collaboration of the majority of hospitals in the Netherlands guarantees high-quality research while minimising the workload for clinicians.

Many RCTs have been completed and resulted in interesting insights, not seldom against general expectations. Some simple interventions turn out to be surprisingly effective. For example, when women are immobilised for 15 minutes after intra-uterine insemination, they have a 50% increased chance of pregnancy from 20% to 30% after four cycles.[7] On the other hand, sometimes, well intended and reasonable interventions are proven to be ineffective. Prolonged tocolysis in case of threatened preterm labour, for example, does not improve perinatal complications,[8] and conservative surgery that aims to maintain the fallopian tube in case of tubal pregnancy does not result in higher pregnancy rates as compared with radical surgery. Recently, the ProTwin trial showed a massive 50% reduction of preterm birth and perinatal morbidity and mortality in short cervix twin pregnancies by the use of a simple pessary. These concrete contributions to world-wide medical care resulted from mutual effort.

Potentially, millions if not billions of Euros are spent on ineffective and therefore by definition useless treatments. This generates a burden to patients or even complications and a worsened health outcome. In order to decide which treatments are worth spending our limited health care budget on and which are not, scientific evaluation is a prerequisite.

MULTIDISCIPLINARY RESEARCH

Until recently, trials conducted by the consortium were limited to obstetrical or gynaecological issues. However one of the most challenging features of the obstetric patient population is that it involves many pre-existing diseases requiring a multidisciplinary approach. Such is the case for pregnant women with diabetes. The consortium extended its playing field by initiating a multidisciplinary multicentre RCT on this specific patient group. Although the road for obstetric RCTs is paved,

side roads connecting other disciplines are still a bit bumpy. With the introduction of the GlucoMOMS trial, which evaluates the effect of additional use of Continuous Glucose Monitoring on pregnancy outcome, several challenges came across.

The way the national consortium structured research and integrated it in daily practice is unique to obstetrics/gynaecology. Every specialist knows how the organisation operates and how to optimally receive assistance from it. Naturally, the organisation is new to endocrinologists and may appear unduly assertive. Fortunately, in the project group of the trial both disciplines are represented and we should be able to overcome such challenges.

GLUCOMOMS

Despite the above-mentioned challenges, the project group of the GlucoMOMS trial still strongly feels implementation of an expensive tool as CGM in the routine care of patients during a specific, temporarily condition (pregnancy) should be supported by scientific proof on (cost-) effectiveness. Providing an intervention during the course of pregnancy must have an evident positive effect on pregnancy outcome.

Meanwhile, two RCTs have been published on effectiveness of CGM use on pregnancy outcome. Murphy *et al*. evaluated the additional use of intermittent retrospective CGM in 71 women with type 1 or 2 diabetes mellitus (DM). The incidence of macrosomia (birth weight >90th percentile) was significantly lower in the CGM group, 35% as opposed to 60% in the control group. Other pregnancy outcome measures did not differ.[9] Secher *et al*. randomised 154 women with type 1 or 2 DM for either additional intermittent use of RT-CGM or standard care. No difference was found in the prevalence of macrosomia or other pregnancy outcome measures, as well as in HbA_{1C}.[10] Hence, a definite conclusion cannot yet been drawn. The GlucoMOMS trial may further clarify these opposing results.

Although initiated by the Dutch obstetric consortium, it must be stressed that it is not intended to invade the professional autonomy of diabetes specialists. Endocrinologists and gynaecologists together bear responsibility for this particular patient group.

When contacting different endocrinology departments throughout the country to discuss participation in the GlucoMOMS trial, some interesting points of view were shared. Some examples underline the problems which currently exists:

'It's a dilemma: do we dare to subject a treatment strategy, which we find ourselves strongly believing in, to scientific evaluation?'

'In principal, we gladly participate in national trials, however this trial is in conflict with our daily practice, since all of our pregnant patients with DM type 1 are offered CGM'.

'Personally, I regret not participating in the national trial, scientific evaluation is important, but I fear practice has sailed passed science. Unfortunately this happens more often without evidence on cost-effectiveness'.

'Due to high morbidity and mortality in diabetic pregnancies, it is essential to pursue normoglycaemia during these pregnancies. Before the introduction of CGM, this was nearly impossible. CGM is now reimbursed for diabetic pregnancy and therefore I feel it is unethical not to offer my patients CGM'.

'This is very complicated. The reality is that evidence-based medicine is passed by the health care insurer (most of the time it is the other way around, as it should be), however, medical tools deal with other playing fields when it comes to reimbursement than does medication'.

'We can hardly get away with it, since many articles on CGM can be found on the internet to which patients refer'.

'Our hospital will not participate in the national trial because we already provide CGM for all pregnant patients with DM type 1'.

Current medical practice is filled with treatments based on faith rather than exact scientific proof. Doctors prefer offering their patient (un-evaluated) treatment options to offering nothing or even worse: the unpopular truth of 'we don't know what's the best thing to do'. Although intentions are evidently sincere, doctors should be critical. Doctors decide the appropriate treatment option and this should be done independently from the reimbursement status (instead of offering it for the mere fact that it is there to offer). Furthermore, a doctor should contribute to scientific evaluation in order to improve the quality of our profession and health care in general.

POTENTIAL IMPROVEMENTS

By reflecting on the current events regarding the GlucoMOMS trial we hope to motivate our colleagues to accept the fact that current evidence on CGM use in pregnancy is limited and to comprehend that by collaborating, indispensable evidence is within reach.

Regular multidisciplinary meetings to discuss diabetic pregnant patients should be held in every hospital treating such patients, in order to improve insight into each other's professional considerations. This may also facilitate the participation in national or international trials.

Furthermore, providing insight into treatment strategies and clinical outcomes may also help to map out current practice and evaluate national discrepancies.

CONCLUSION

Improving diabetic pregnancy outcome is a goal shared by endocrinologists and obstetricians. The best result will come from collaborating not only in the doctor's office but also in critical evaluation of our treatment strategies by means of randomised clinical trials. By providing a scientific basis for medical interventions we justify our current practice and enable rational future reimbursement policies.

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LETTER TO THE EDITOR

Cost-minimisation in vitamin B12 deficiencies: expensive diagnostics can reduce spending

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To the Editor,

The diagnostic approach to detect vitamin B12 deficiencies is centred around measuring plasma vitamin B12 concentrations, even though these do not always give a correct representation of the functional availability of vitamin B12. For example, markers of functional vitamin B12 deficiencies, such as methylmalonic acid (MMA), have been shown to be aberrant in only 30% of patients with low-normal vitamin B12 concentrations (between 100 and 200 pmol/l).[1] As such, guiding therapeutic intervention by MMA instead of vitamin B12 will prevent unnecessary treatment in patients with indecisive (low-normal) vitamin B12 concentrations, in whom B12 measurements are not conclusive in determining deficiencies.

In the Netherlands, treatment generally consists of intramuscular (IM) administration[2] of vitamin B12, although the clinical effectiveness of high-dose oral supplementation (OS) was shown in various prospective studies.[3,4] As a small amount (±1%) of vitamin B12 is absorbed by passive diffusion, without the mediation of intrinsic factor,[5] OS is also effective in patients with deficiencies in the active uptake of vitamin B12. Hereby, daily oral administration of 1000 μg of vitamin B12 is considered to be sufficient for treating deficiencies (reviewed by Andres *et al.*[6]).

To investigate which combination of diagnostic and therapeutic options for vitamin B12 deficiencies allows the lowest cost, and hence, the most efficient care provision, we applied a cost-minimisation analysis to a commonly used diagnostic flowchart (adapted from Wiersinga *et al.*[7]). The diagnostic flow chart and the resulting costs in the first year of treatment are shown in *figure 1*.

In patients with plasma vitamin B12 concentrations between 100 and 200 pmol/l, the MMA-guided IM treatment saves approximately € 91 per person per year in the first year of treatment (PPPY) compared with direct IM treatment. OS treatment enables the additional saving of approximately € 39 PPPY. Guiding OS by MMA prevents unnecessary treatments at roughly the same cost of direct OS.

In summary, the additional diagnostics prevent unnecessary treatment and our calculations present a clear example of how laboratory diagnostics can be used to improve both patient wellbeing and reduce healthcare spending. Moreover, the use of MMA analysis to guide the diagnosis and treatment of vitamin B12 deficiencies enables substantial reductions in costs. The greatest efficiency in care is obtained by combining MMA analysis and OS treatment. Unfortunately, Dutch health insurance companies only reimburse IM treatment. Time to reconsider their policy?

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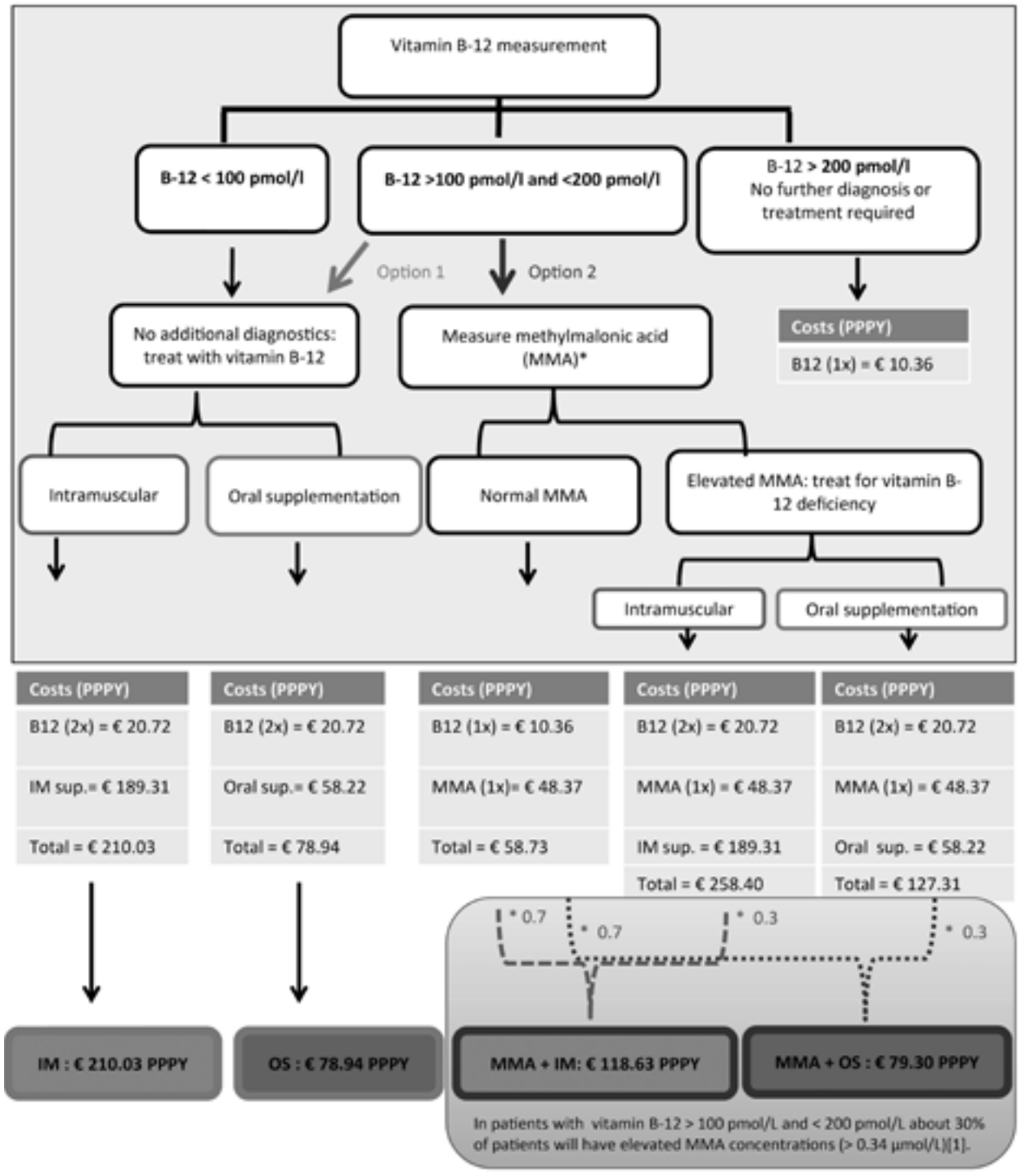
Figure 1. *Diagnosis of vitamin B12 deficiencies and the associated costs per person per year (PPPY) in the first year of treatment. Costs taken into account are: laboratory analysis of MMA and vitamin B12, vitamin B12 medication (IM or OS), pharmacy dispensing fee and administration of injections (by general practitioner). Costs and resources were gathered from the rates published in 2011 or 2012 by the Dutch Healthcare Authority (www.nza.nl)*



Assumptions: IM treatment consists of 10 injections in the first months, followed by 1 injection every 2 months (as advised by the Dutch college of General Practitioners).[2] OS treatment consists of 1 (1000 µg) vitamin B12 tablet per day.[6] Costs of vitamin B12 tablets for OS treatment: 100 tablets (1000 µg each) € 15.95 (obtained from an internet search, this price seems representative for online and offline alternatives)

LETTER TO THE EDITOR

Strongylodiasis in a mine worker

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To the Editor,

Papendorp *et al*. described a patient with disseminated *Strongyloides stercoralis* hyperinfection after steroid use.[1] The case underscores the necessity of a complete travel history before starting high-dose steroids. I would like to point out that *Strongyloides* has also been endemic in areas outside the tropics and that just a travel history is insufficient.

At the former St. Jozef Hospital in Kerkrade (in the southern part of the Netherlands) we treated a 74-year-old man with intensive chemotherapy for a non-Hodgkin lymphoma in 1993. During this treatment the patient developed a disseminated *Strongyloides stercoralis* infection with microscopically confirmed larvae in the sputum.

He had never travelled to or lived in a tropical area. He was born in Poland and came as a young man to the Netherlands and had stayed all his life. However, he did work for many years in the Dutch coal mines. As the older staff physicians know, infection with *Strongyloides* was quite common in the coal mines. The conditions in the coal mines were favourable for *Strongyloides* due to the high temperature and humidity.

Although the last mines closed in 1974, there are still many elderly people who once worked in the mines and strongylodiasis should be kept in mind.

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INFORMATION FOR AUTHORS

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