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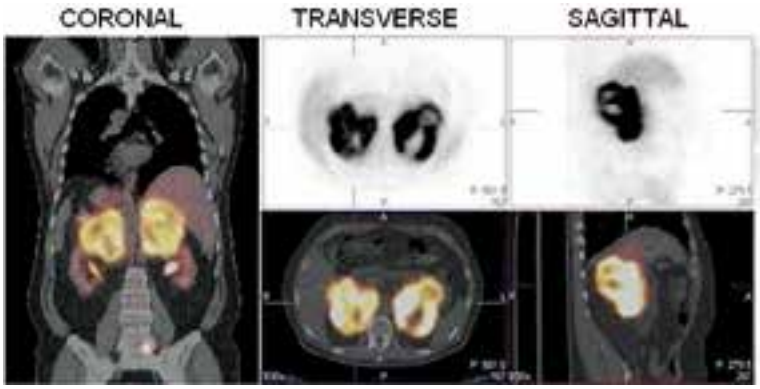

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Contents

EDITORIAL

New oral anticoagulants versus vitamin K antagonists in countries with good INR control

M. Coppens

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Routine monitoring of the international normalised ratio (INR) in patients treated with vitamin K antagonists (VKAs) is mandatory because of a narrow therapeutic index combined with an unpredictable and highly variable anticoagulant effect. The lowest risk of thrombotic and bleeding complications is reached by maximising the time in the therapeutic range (TTR). A low TTR indicates poor INR control and has been associated with increased risks of thrombotic and bleeding complications in patients with atrial fibrillation (AF).[1,2] The TTR is determined by individual characteristics, such as use of co-medication, as well as by the centre that manages the patient.[1] The mean TTR per centre (cTTR) thereby reflects the quality of management of VKA therapy of that specific centre.

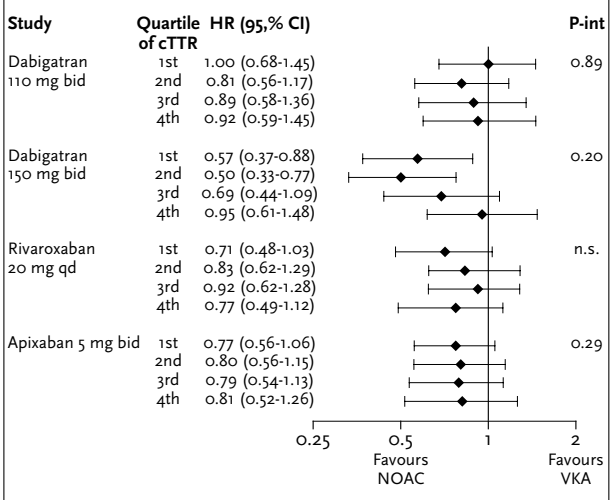
The new oral anticoagulants (NOACs) dabigatran etexilate, rivaroxaban and apixaban have been compared with VKA therapy in over 50,000 patients with AF.[3-5] The NOACs are used at a fixed dose without the need for routine coagulation monitoring and offer significant simplification of anticoagulant therapy. The results of the AF trials indicate that unmonitored NOACs are either non-inferior or superior compared with monitored VKAs. Concern has arisen if the benefits observed in the AF trials will apply in countries with a high quality of INR control of VKA therapy after the publication of a subgroup analysis of the RE-LY trial in 2010.[6] Although there was no significant interaction between the cTTR and treatment for the prevention of stroke or systemic embolism, the hazard ratio (HR) in the upper quartile of cTTR (>72.6%) of dabigatran etexilate 150 mg twice daily *vs* warfarin suggested a loss of superiority of dabigatran etexilate in centres with the highest TTR (HR 0.95, 95% confidence interval 0.61-1.48).[6]

Management of VKA therapy in the Netherlands is provided by a nationwide network of thrombosis services. In their annual reports, the percentage of INR results within the therapeutic range is consistently 70-80%.[7] However, the cross-sectional method used to calculate this percentage differs from the widely used Rosendaal method.[8] Moreover, the Dutch Federation of Thrombosis Services uses a wider therapeutic range (INR 2.0-3.5) than the therapeutic range in randomised controlled trials (INR 2.0-3.0). It is therefore hard to compare the quality of INR control in the Netherlands with the cTTRs from the trials. In this issue of the Netherlands Journal of Medicine, Bezemer and colleagues compare the Dutch cross-sectional method with the Rosendaal method in a representative sample of patients treated with VKA therapy in the Netherlands.[9] The results show that the two methods produce similar results with a TTR of 75% for the therapeutic range of 2.0-3.5. The TTR for the narrower INR target range of 2.5-3.5 was 60%. This study is the first to report the quality of INR control achieved in the Netherlands in terms that can be compared with international standards. The study shows that the quality of management of VKA therapy in the Netherlands is good, but not the best worldwide.

How should these results influence the expectations of the potential benefits of NOACs over VKA therapy in the Netherlands? Over two years have passed since the initial subgroup analyses by cTTR from the RE-LY trial. Similar analyses have now been presented for the two other atrial fibrillation trials, which allow a revaluation of the concept that NOACs may not provide the same benefits over VKAs in countries with good INR control.[10,11] The analyses of the three AF trials comparing NOACs with VKAs according to subgroups of cTTR for the primary efficacy outcome of stroke or systemic embolism are presented in *figure 1*. The results show that there is no significant interaction between quartiles of cTTR and treatment. This indicates that the benefits of NOACs over VKA apply to countries with poor INR control as well as in countries with good INR control. Although one may argue that a non-significant trend towards decreased superiority of dabigatran etexilate 150 mg twice daily *vs* VKA is present in centres with the highest cTTR, no such trend is visible

for rivaroxaban, apixaban or the low dose of dabigatran etexilate (*figure 1*). Moreover, the cTTR for the narrower INR range of 2.5-3.5 achieved by the Dutch Federation of Thrombosis Services is lower than the highest quartile of cTTR in each of the AF trials. With these new subgroup data from the AF trials, we should feel confident that most of the benefits of NOACs over VKA therapy will also apply to countries with good INR control including the Netherlands.

Figure 1. *Efficacy of NOACs compared with VKA therapy for prevention of stroke or systemic embolism in patients with AF, according to subgroups of cTTR*



cTTR = the mean time in therapeutic range per centre; HR = hazard ratio of the NOAC vs. VKA therapy; 95% CI = 95% confidence interval; P-int = p value for interaction between quartile of cTTR and treatment; NOAC = new oral anticoagulant; VKA = vitamin K antagonist; bid = twice daily; qd = once daily.

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REVIEW

The long-term outcome of the Kasai operation in patients with biliary atresia: a systematic review

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ABSTRACT

Background: Biliary atresia (BA) is a progressive inflammatory destructive process of the bile ducts occurring in about one of every 20,000 live births. If left untreated, biliary atresia can lead to liver failure. The only effective treatments for BA at the moment are the Kasai operation and liver transplantation. Kasai portoenterostomy increases the survival of children with BA and postpones subsequent liver transplantation. Because long-term survival is rare, there is not much known about the long-term efficacy of the Kasai operation.

Methods: The aim of this review was to study the outcome of patients with BA who survived more than 20 years on their native liver. We performed a systematic search on PubMed using MeSH terms for articles describing the long-term outcomes of patients with biliary atresia. We searched for patients who have lived at least 20 years with their native liver and we registered the number of complications. The endpoints identified in these articles were: death, cholangitis, portal hypertension and gastrointestinal bleeding.

Results: From 53 articles we included 14 articles for analysis. In total 184 patients were above the age of 20 years. Of these 162 patients, 88% (162/184) were still alive with their native liver and 60.5% (98/162) were suffering from liver-related complications.

Conclusions: It is possible for patients with biliary atresia to survive more than 20 years on their native liver after undergoing the Kasai operation during early infancy. However, 60.5% of the long-term survivors alive on their native liver end up suffering from progressive liver-related complications.

KEYWORDS

Biliary atresia/surgery, cholangitis, follow-up, portoenterostomy, survival rate

INTRODUCTION

Biliary atresia (BA) is a progressive inflammatory destructive (obliterative) process of the bile ducts.[1] It is characterised by a perinatal complete obstruction of all or part of the extrahepatic bile ducts and is always associated with abnormalities of the intrahepatic biliary tree.[2] If left untreated, biliary atresia will lead to liver failure. Biliary atresia is a rare condition with a prevalence of about one patient per 20,000 live births; patients are destined for a fatal outcome if they are not treated during the first few months of life. The only effective treatments for BA at present are hepatic portoenterostomy (Kasai operation) and liver transplantation.

The Kasai operation was introduced in 1959,[3] and consists of constructing a new bile drainage system, generally by creating an anastomosis of the jejunum by a Roux-en-Y loop to the porta hepatis region to re-establish a connection between the intrahepatic bile ducts and the intestine. If successful, Kasai portoenterostomy increases the survival of children with BA and consequently postpones subsequent liver transplantation. Studies have reported 20-year survival rates in patients with their native liver of 21%,[4] 25%,[5] 22%,[6] 23%[2] and 44%.[7] However, most long-term survivors develop complications.[8] Until the age of 18, BA patients are generally managed by paediatricians or paediatric surgeons. After the age of 18 these patients are referred to and managed by gastroenterologists. It is

therefore important that gastroenterologists become aware of the fact that in long-term survivors who have undergone the Kasai operation, complications such as recurrent cholangitis and portal hypertension may occur.

The aim of this review is to study liver condition and liver-related complications in patients treated with this operation during early infancy after a follow-up of 20 years while retaining their own liver. This review attempts to give an overview of the most important complications suffered by these patients and the prevalence of these complications.

METHODS

Literature search

The PubMed database was searched on 7 January 2012 using the MeSH term combination:

("Biliary Atresia/surgery"[MeSH Terms] AND ("Follow-Up Studies"[MeSH Terms] OR "Survival Analysis"[MeSH Terms] OR "cholangitis"[MeSH Terms] OR "Portoenterostomy, hepatic"[MeSH Terms])). The limits were set to 'English' and 'Aged 19+ years'.

Selection of studies

Articles were excluded based on title and abstract when research on liver transplantation, non-gastrointestinal complications, diagnostic tools used for follow-up and alternative postoperative treatment were described. Articles that described studies with a population younger than 20 years, articles that did not contain information on BA or the added complications and articles that were not available for Erasmus MC were also excluded.

After reading the articles, some were excluded because the minimum follow-up was shorter than 20 years or the age of the population was unclear. One article contained no information on the complications of biliary atresia. After excluding all these articles, the references of the remaining articles were scanned, which yielded additional relevant articles. Some articles that were retrieved described the same population, thereby creating an overlap. To resolve this issue we included the most recent article that described the whole population.

Outcome

Using the articles retrieved from the systematic review we defined a cohort of patients that survived for 20 years after a hepatoporto-enterostomy with their own liver. Within this cohort the number of patients who died or underwent a liver transplant after they had reached 20 years of age and the number of patients who are still alive with their native liver were described. We also retrieved information on complications in patients aged 20 years or older, who are still alive with their native liver after 20 years of age as the secondary outcome. The complications described in this article are: cholangitis, portal hypertension and gastrointestinal bleeding.

Data analysis

We extracted the study characteristics and the primary and secondary outcome measures from each article. The study characteristics included the authors, year, journal of publication, country of study and population above 20 years of age. For each outcome (death, alive with/without liver transplantation, with/without complications, cholangitis, portal hypertension, gastrointestinal bleeding or hepatocellular carcinoma) separately, we counted the number of patients in each article, summed them up and displayed them in a table or graphic.

RESULTS

The literature search and selection of studies

The results of our systematic literature search and our study selection is shown in a flowchart (*figure 1*). From the literature search we identified 53 studies, from which

Figure 1. *Results of systematic literature search and study selection*

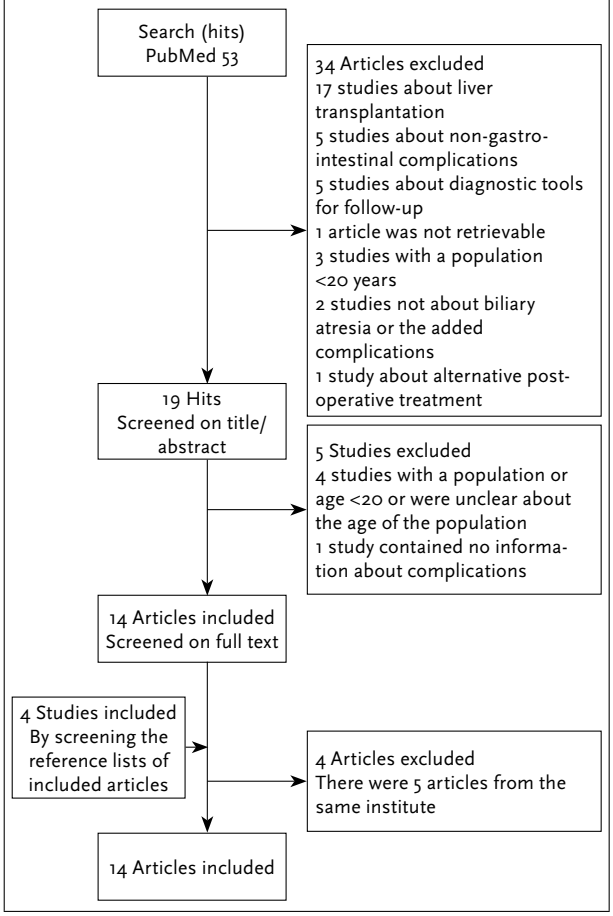

Table 1. *Characteristics of the articles*

Source	Publication date	Journal	Country of study	N alive at 20 years with native liver
Lykavieris et al.[2]	2005	Hepatology	France	63
Shinkai et al.[7]	2009	J Pediatr Gastroenterol Nutr	Japan	35
Nio et al.[6]	1997	Tohoku J Exp Med	Japan	30
De Vries et al.[5]	2011	Clin Gastroenterol Hepatol	Netherlands	28
Toyosaka et al.[14]	1993	J Pediatr Surg	Japan	6
Shimizu et al.[11]	1997	Tohoku J Exp Med	Japan	6
Pintér et al.[1]	2004	J Pediatr Surg	Hungary	4
Watanabe et al.[15]	1997	Tohoku J Exp Med	Japan	3
Hung et al.[3]	2006	J Pediatr Gastroenterol Nutr	Taiwan	2
Takahashi et al.[13]	2009	J Pediatr Surg	Japan	2
Raffensperger[9]	1991	J Pediatr Surg	US	2
Hol et al.[8]	2008	Eur J Gastroenterol Hepatol	Netherlands	1
Kasai et al.[12]	1988	J Pediatr Surg	Japan	1
Yamanaka et al.[16]	2005	J Pediatr Surg	Japan	1

34 studies were excluded based on title and abstract. We read the full text of 19 articles from which five articles were excluded. We screened the reference lists of the 14 remaining articles; this resulted in four additional articles. Finally, we excluded four articles because they contained an overlap in population. Thus, 14 articles were included for further analysis.

Characteristics of the articles

Of the 14 articles included, seven described a follow-up study, three articles described a retrospective cohort study and four articles were case reports. Of the 14 studies, eight studies are from Japan. There are ten studies that described very few or only one patient. Four articles described a larger patient group (*table 1*).

Population

The 14 studies included a total group of 184 patients above 20 years of age. In this group, eight patients died and 14 were alive with liver transplantation after 20 years of age. There were 162 patients living with their native liver (*table 2*). All patients who died or had undergone liver transplantation had end-stage liver disease or suffered from severe liver-related complications.

Complications

Of the 162 patients who are alive with their native liver, 39.5% (64/162) are alive without complications. The remainder of the patients have developed complications (*table 2*).

All the patients who developed complications had experienced episodes of cholangitis. Of the 98 patients with cholangitis, 80% (78/98) developed portal hypertension. Of the patients with portal hypertension, 45% (35/78) experienced gastrointestinal bleeding. In one patient a hepatocellular carcinoma was found (*table 2*).

Table 2. *Analysis of the population*

	Number
Total population >20 years	184
Death	8 (4.3%)
Alive with Ltx	14 (7.6%)
Alive without Ltx	162 (88%)
Without complications	64 (39.5%)
With complications	98 (60.5%)
Cholangitis	98 (100%)
Portal hypertension	78 (80%)
Gastrointestinal bleedings	35 (45%)
Hepatocellular carcinoma	1 (1.3%)

Ltx = liver transplantation.

DISCUSSION

This systematic review shows that there are patients born with biliary atresia who can survive for more than 20 years with their native liver. This review included 184 patients of which 60.5% of the patients eventually developed severe complications such as cholangitis, portal hypertension, gastrointestinal bleeding and hepatocellular carcinoma. One can assume that in the end, most of these patients will need to undergo a liver transplantation.

It was not possible to calculate a survival rate because not all the included articles were follow-up studies, other included articles did not provide information on deceased and transplanted patients before the age of 20. Earlier publications have described 20-year survival rates on a native liver, which varies from 21% to 44%.[2,4-7]

The importance of these findings can be *extrapolated to clinical practice* where until the age of 18 these patients are generally managed by a paediatrician or paediatric

surgeon. After the age of 18 most of these patients are managed by the gastroenterologist. It is especially necessary for the gastroenterologist to realise that in long-term survivors after Kasai operation developing recurrent cholangitis and portal hypertension liver transplantation should be considered early to avoid death to liver-related mortality. This review shows that the majority of these patients have severe liver disease that requires close follow-up.

Almost all the articles we included for this review describe only a small population of BA patients. This is why we also took case reports into account. Some case reports described detailed outcomes that were not available in other articles. When analysing the complications, it became difficult to understand which patients suffered from which complications in the articles. To avoid overlap in patients who had more than one complication we assumed the following: cholangitis led to portal hypertension which led to gastrointestinal bleeding. That means that we assumed that patients who had portal hypertension were the same patients who suffered from cholangitis.

Most of the articles we analysed were publications from Japan. The Kasai operation was introduced in Japan in 1959 and at first was probably only used in Japan to treat biliary atresia. It only became accepted in America[9] and the Netherlands[10] in around 1970. Before that, Japan was practically the only country using the operation to treat BA. This could be the reason why Japan has more long-term survivors.

We would suggest that more research is needed on the further development of the disease after 20 years of survival on a native liver. It could be a great asset to the treatment of biliary atresia if research was done to assess whether patients should be added to the liver transplantation waiting list after clinical manifestation of cholangitis.

Altman *et al.* provided an important insight into survival rates in patients with biliary atresia.[4] However, due to the fact that suffered complications were not described, this article had to be excluded from this review.

Taking everything into account, we can conclude that there is an extensive lifelong follow-up needed in these patients, so complications can be identified and the optimal treatment can be initiated.

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REVIEW

Obesity-associated low-grade inflammation in type 2 diabetes mellitus: causes and consequences

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ABSTRACT

The epidemic of overweight and obesity is a major problem because of the plethora of health and economic issues that it induces. Key among these is the sharply increasing prevalence of type 2 diabetes (T2D) and cardiovascular disease. The development of T2D is characterised by two processes: 1) insulin resistance, resulting from impaired insulin signalling and leading to an increased demand for insulin, which must be met by increased insulin production by pancreatic β-cells (compensatory β-cell function); and 2) β-cell dysfunction, with T2D developing when the amount of insulin that is produced is insufficient to meet the demand. Overweight and obesity, especially in case of abdominal fat accumulation, are associated with systemic low-grade inflammation. This low-grade inflammation is characterised by, among other things, higher levels of circulating proinflammatory cytokines and fatty acids. These can interfere with normal insulin function and thereby induce insulin resistance, and have also been implicated in β-cell dysfunction. This review focuses on the known and emerging relations between inflammation and T2D. We first discuss current views on the effects of fat distribution on adipose tissue inflammation and adipose tissue dysfunction. Next we focus on the detrimental roles of proinflammatory cytokines and fatty acids on insulin signalling and β-cell function. In the last part of this review we provide some insight into novel players in (the initiation of) inflammation in overweight and obesity, and their effects on T2D and vascular dysfunction.

KEYWORDS

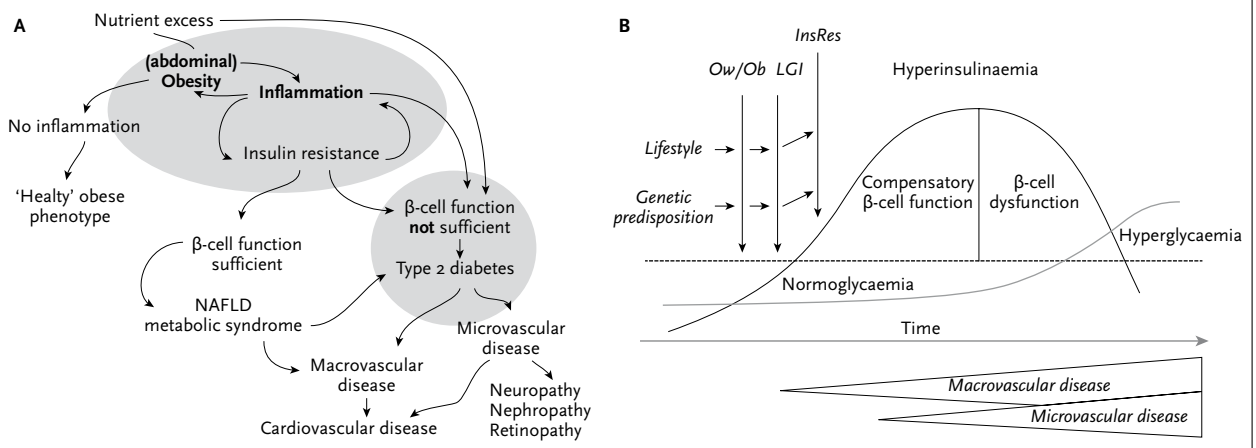
Obesity, insulin resistance, β-cell dysfunction, vascular dysfunction, innate and adaptive immunity

HOW DOES OBESITY CAUSE TYPE 2 DIABETES?

The epidemic of overweight and obesity has caused a dramatic increase in the number of individuals with metabolic abnormalities and premature cardiovascular disease (CVD). The prevalence of diabetes, and especially of type 2 diabetes (T2D), which comprises 80-90% of all individuals with diabetes, also rises sharply with the obesity epidemic. Two processes contribute to the development of T2D. Impaired insulin signalling – also known as insulin resistance – leads to an increased demand for insulin and this increased demand must be met by an increased insulin production by the pancreatic β-cells, a process known as compensatory β-cell function. Thus, obesity-induced insulin resistance will initially lead to higher circulating insulin concentrations but in case of prolonged and/or worsening insulin resistance, β-cells may no longer be able to meet the high demand. This will eventually lead to insufficient hepatic and peripheral glucose disposal, subsequently to higher circulating levels of glucose and eventually to the development of T2D (***figure 1***). In the past three decades, both CVD and diabetes, in particular obesity-induced T2D, have been recognised as inflammatory diseases. The systemic low-grade inflammatory response that is often observed in obesity detrimentally affects both insulin signalling and β-cell function and may thus contribute to the development of T2D.

At the population level, the relative risk of developing T2D rises sharply with an increase in body mass index [BMI), as a measure of excessive body fat. However, within a narrow range of BMI levels, individuals can vary enormously with respect to insulin resistance, and this inter-individual difference has been attributed, to an important extent, to differences in the distribution of fat over the body.[1] In

Figure 1. *Development of insulin resistance and B-cell failure are involved in the development of obesity-associated T2DM*



Panel A presents the main events that underlie the development of obesity-associated T2DM and the two main metabolic hubs that are involved, i.e. development of insulin resistance and the development of β-cell failure. Caloric intake in excess of energy expenditure leads to the accumulation of fat. If this fat accumulates primarily in the superficial subcutaneous adipose tissue depot, the low-grade inflammatory response will likely be minimal to absent. If, however, due to genetic and/or lifestyle factors, accumulation of fat is shifted towards the abdominal fat and ectopic depots, a persistent low-grade inflammatory response will develop. This low-grade inflammatory response will lead to cellular insulin resistance and also attract proinflammatory immune cells to adipose tissue, which can worsen the inflammatory response. Insulin resistance increases the demand for insulin, but as long as the pancreatic β-cells can respond with a sufficient compensatory insulin production, this will lead to a state of normoglycaemia with hyperinsulinaemia, which is often associated with dyslipidaemia, hypertension and further ectopic fat accumulation. If, however, due worsening of the insulin resistance and, again, to individual genetic and/or lifestyle factors, the secretion capacity of the β-cells is no longer sufficient, hyperglycaemia and hence T2D will develop

Panel B represents the timeline of these events. Genetic and lifestyle factors most likely determine not only the development of (abdominal) obesity (Ow/Ob), low-grade inflammation (LGI) and insulin resistance (InsRes) but also the time scale (years or decades) it takes to progress through the different stages of the development of obesity-associated T2D. The major vascular complications of T2D, i.e. macrovascular and microvascular disease are each presumed to start prior to the development of hyperglycaemia

particular, at the same BMI, more upper body fat (also referred to as abdominal or central obesity), as represented by a higher waist circumference or higher waist-to-hip ratio, has been found to be associated with a higher risk of T2D compared with less upper body fat.[2,3]

Several different fat depots have been identified, each with specific physiological and metabolic functions. Subcutaneous fat is the largest fat depot in the human body and comprises approximately 70-80% of total body fat. The second largest fat depot is visceral fat, which comprises approximately 10-15% of total body fat.[4] The subcutaneous fat depot should probably not be regarded as functionally homogeneous. For example, it may be divided into peripheral versus central subcutaneous fat, which were shown to have specific and sometimes contrasting metabolic functions.[5-7] Another way to identify metabolically distinct parts of the subcutaneous fat depot is to divide it into superficial and deep subcutaneous fat. This distinction appeared to be particularly relevant for abdominal subcutaneous fat, where the deep subcutaneous depot appeared to behave metabolically more similar to visceral than to superficial subcutaneous fat.[8]

Visceral (sometimes referred to as abdominal and/or omental) fat is generally considered the 'bad' fat depot. Adipocytes within the visceral fat depot show substantially higher fatty acid fluxes than superficial subcutaneous abdominal adipocytes.[9] These non-esterified fatty acids (NEFA), often referred to as free fatty acids, can contribute to insulin resistance and β-cell failure (see below). Visceral fat is characterised by higher secretion of proinflammatory cytokines such as tumour necrosis factor (TNF)-α and interleukin (IL)-6 and lower secretion of adiponectin, the anti-inflammatory adipokine, as compared with abdominal subcutaneous fat.[10,11] Both visceral and deep subcutaneous fat were shown to be associated with insulin resistance.[4,12-15]

In addition to these main fat depots, there are additional fat depots that are usually referred to as 'ectopic' fat. These additional fat depots are considerably less important in volume, but appear highly relevant with respect to regulatory and metabolic functions. Generally, ectopic fat depots are larger in individuals who have a more central fat distribution with a relatively large amount of visceral fat. Ectopic fat includes, for example, perivascular as well as epicardial and pericardial fat depots – which are relatively small but distinct patches of fat around the vasculature and the heart – and intramuscular and intrahepatic fat – which is the accumulation of triglycerides within muscle and liver, respectively.

The adipose tissue depots that are in close proximity to the vasculature and the heart have been implicated in the development of vascular dysfunction,[16] probably via locally produced mediators that can contribute to a local

inflammatory response[17,18] as well as to local insulin resistance, which may directly affect vascular function[19,20] and might as such contribute to hypertension and CVD (see also below).

Intramuscular fat is mainly derived from the circulation and the amount of fat that is accumulated in muscle was associated with whole body insulin resistance.[21] Indeed, T2D patients were shown to have more visceral and intramuscular fat than non-diabetic controls.[22] Moreover, intramyocellular triglyceride content in the soleus muscle was 40% higher in offspring of T2D parents than in control subjects,[21] suggesting that increased intramuscular fat may precede and contribute to the development of T2D.

The main sources of hepatic fat are endogenous fatty acids, which are newly synthesised in hepatocytes, and (diet and adipose tissue derived) exogenous fatty acids. Fat accumulation in the liver (mainly as triglycerides) is currently considered an important risk factor for metabolic and cardiovascular diseases. Uncomplicated hepatic fat accumulation (steatosis) is the first stage of the full spectrum of non-alcoholic fatty liver disease (NAFLD). NAFLD may progress from simple steatosis to steatohepatitis, fibrosis and eventually liver cirrhosis. Obesity and obesity-associated T2D are mostly associated with the earlier NAFLD stages (steatosis, steatohepatitis), although also late stages of NAFLD i.e. liver cirrhosis, were shown to be associated with a high prevalence of T2D.[23] The 'portal theory' is the concept that, with an increasing amount of visceral fat, the liver is exposed to higher concentrations of proinflammatory cytokines and NEFA that are released from the visceral fat depot and directly transported, via the portal vein, to the liver where they contribute to the development of NAFLD.[24] Thus, the occurrence and severity of visceral fat accumulation and NAFLD are highly correlated and inflammatory changes in visceral adiposity and NAFLD are aetiologically intertwined. Hence, it is not easy to dissect their independent contributions to the development of obesity-associated T2D, especially in humans. The general view is that NAFLD adversely affects insulin resistance and the risk of T2D and CVD.[25] The visceral fat depot, in turn, is highly relevant in an aetiological sense, as it precedes and induces the development of NAFLD and other ectopic fat depots. In addition, visceral fat may contribute directly to systemic low-grade inflammation and increased systemic levels of NEFA.

But it appears that not all fat is bad. It has consistently been shown that approximately 25-30% of obese individuals do not develop insulin resistance; these are the so-called healthy obese. There is also accumulating evidence that expansion of the fat depot(s) will not by definition lead to an inflammatory response and insulin resistance. Efficient expandability of the superficial subcutaneous fat depot, through e.g. intrinsic genetic properties and/or an attenuated inflammatory response, is likely to improve flexibility to process excess caloric intake with limited triglyceride overflow into the visceral and ectopic fat depots. A large capacity for storage of triglycerides in the superficial subcutaneous, metabolically less active fat depot, may thus result in less 'overflow' of triglycerides into the deep subcutaneous and visceral fat depots.[26,27] Very recently it was indeed shown that upon feeding healthy men a high-fat diet, accumulation of fat in the visceral fat depot was highest in those subjects who had the lowest expression of lipid storage-related genes in their subcutaneous fat.[28] The possibility that subcutaneous adipose tissue function and/or inflammation may contribute to redistribution of fat towards the visceral depot is also corroborated by recent data that infiltration of macrophages into human abdominal superficial subcutaneous adipose tissue was associated with larger visceral fat depots.[29] Accordingly, the expression of inflammation-related genes was significantly upregulated in abdominal subcutaneous adipocytes of obese, as compared with non-obese individuals.[30,31] In line with these data, we recently showed that preadipocytes isolated from subcutaneous adipose tissue of T2D patients had a gene expression profile that was consistent with a decreased differentiation capacity.[32] In animal models subcutaneous fat expansion could, for example, be achieved by fat-specific overexpression of adiponectin in genetically obese mice, which resulted in increased peripheral obesity but less accumulation of ectopic fat (visceral, liver, muscle) with significant improvement in insulin resistance. Adiponectin-overexpressing mice showed an increased expression of peroxisome proliferator-activated receptor (PPAR)-γ target genes and, despite massive obesity, had few macrophages in their fat depots, concomitant with lower plasma IL-6 and TNF-α levels.[33] Notably, recent data show that adiponectin can exert part of its anti-inflammatory effects on adipose tissue via regulation of microRNAs that can suppress intracellular proinflammatory pathways, such as toll-like receptor (TLR)-4 signalling (see below).[34] MicroRNAs comprise a promising new field of potential novel treatment targets for insulin resistance and T2D, because they appear to have a vast functional and regulatory capacity, also in other pathways that may contribute to insulin resistance and T2D.[35] PPAR-γ activation by rosiglitazone in mice was also associated with higher body weight and adipose tissue expansion, but with less accumulation of fat in the liver. In these mice a higher macrophage infiltration into adipose tissue was seen, but these were primarily alternatively activated (M2) macrophages that are considered to have anti-inflammatory capacities (see below), and their presence was associated with ameliorated insulin resistance.[36]

Together, current data suggest that visceral/omental, abdominal deep subcutaneous, as well as ectopic fat depots appear to be the culprit fat depots with respect to the generation of an inflammatory response and insulin resistance. There may, however, very well be underlying

metabolic characteristics of the (superficial) subcutaneous fat depots that contribute to the size of these visceral/omental depots. Prevention of adipose tissue dysfunction, of (visceral) fat inflammation, and of ectopic fat deposition may therefore all help to maintain a metabolically healthy obese phenotype.[37]

Obesity – fat distribution | Key points:
- Obesity is strongly associated with T2D
- Visceral, abdominal deep subcutaneous, and ectopic fat were all shown to be associated with an adverse metabolic phenotype
- Subcutaneous fat, especially of the lower body, may have metabolically beneficial functions
- Better capacity for triglyceride storage in adipocytes of superficial subcutaneous fat may prevent overflow of triglycerides into the metabolically unfavourable fat depots

HOW DOES OBESITY CAUSE CHRONIC INFLAMMATION?

It is currently well-accepted that that obesity promotes a state of chronic low-grade inflammation,[38-40] which is reflected not only by an increased production of cytokines and proinflammatory adipokines by adipose tissue, but also by a cellular component. Adipose tissue is heterogeneous in composition and contains, besides mature adipocytes, also immature adipocytes (preadipocytes), endothelial cells, fibroblasts, macrophages and other immune cells. Adipose tissue macrophages are largely bone marrow derived and their number is increased in obesity.[41] Thus, local production of chemoattractants that enhance the homing of monocytes to adipose tissue depots can contribute to adipose tissue inflammation. Macrophages in adipose tissue are overrepresented around dead or dying adipocytes, thereby forming so-called crown-like structures.[42,43] This suggests that adipocyte necrosis may underlie the proinflammatory response and macrophage attraction, but at present their concomitant presence represents an association and a direct causal relation remains to be established.[44]

Accumulation of abdominal fat can induce inflammation via several mechanisms. For example, caloric intake in excess of energy expenditure will lead to expansion of adipose tissue and adipocyte hypertrophy, which may be associated with local hypoxia and adipocyte apoptosis, which in turn generate signals to recruit macrophages.[45] Hypertrophic adipocytes begin to secrete low levels of TNF-α, which stimulate preadipocytes and endothelial cells to produce monocyte chemotactic protein (MCP)-1 (also known as CCL2).[46] Indeed, in a study of monozygotic twins it was shown that *acquired* obesity is characterised by adipocyte hypertrophy and increased expression of the macrophage marker CD68 and TNF-α in subcutaneous abdominal adipose tissue.[47] These proinflammatory changes in acquired obesity were associated with an increase in insulin resistance.[47] In addition to proinflammatory effects induced by local hypoxia, the high rate of protein synthesis during adipose tissue expansion may lead to accumulation of unfolded or misfolded proteins and hence to endoplasmic reticulum (ER) stress,[48-50] which may then also contribute to the production of inflammatory and chemotactic signals.

The exact signals from adipose tissue that initiate macrophage infiltration have not yet been identified. In obesity, TNF-α production is increased in both the adipocyte and the macrophage fraction of adipose tissue and an increase in MCP-1/chemokine (C-C motif) ligand (CCL2) that may be induced by TNF-α has been proposed as primary macrophage attractant[51,52] although these data are not fully consistent.[53] Recently it was shown that TNF-α also induces the production of CXCL5, which is a strong chemoattractant for macrophages. Moreover, mice that were knock-out for the receptor for CXCL5 (i.e. CXCR2) or treated with anti-CXCL5 were less insulin resistant.[54]

Macrophages that are located within the adipose tissue may be pro- or anti-inflammatory, depending on their activation status.[55] Classically activated macrophages (referred to as M1 macrophages) are considered proinflammatory, and the M1 status is induced by, among others, TNF-α and lipopolysaccharides (LPS). Alternatively activated macrophages (referred to as M2 macrophages), on the other hand, primarily function to resolve or dampen the M1-induced inflammatory response and are therefore considered anti-inflammatory. The M2 status is induced by, among others, IL4 and IL10. Although the M1 versus M2 status is a gradient rather than a black-and-white phenomenon, macrophages present in adipose tissue in obesity appear to be predominantly polarised towards the M1 phenotype.[56]

Obesity – chronic low-grade inflammation | Key points:
- Adipocyte hypertrophy, hypoxia and stress may all be involved in adipose tissue inflammation via induction of pro-inflammatory cytokines, as well as of chemokines that attract macrophages
- Adipose tissue macrophages may have a pro-inflammatory (M1), an anti-inflammatory (M2), or an intermediate phenotype, depending on the activating cytokines that are present
- In obesity, macrophages in adipose tissue were shown to be mainly M1

HOW DOES INFLAMMATION CAUSE INSULIN RESISTANCE?

Insulin resistance is a state in which the sensitivity of target cells to insulin, especially with regard to its metabolic actions, is reduced. Inflammatory cytokines, with TNF-α and IL-6 as most extensively studied examples, can directly induce insulin resistance and the level of insulin signal transduction, by using a physiological negative feedback mechanism of normal insulin signalling.[57] Binding of insulin to its functional receptor induces autophosphorylation of tyrosine residues on the intracellular part of the receptor. In the so-called metabolic pathway of insulin signalling, the insulin receptor substrate (IRS), docks the insulin receptor and is trans-phosphorylated in its tyrosine residues via the kinase activity of the phosphorylated insulin receptor. Subsequently, more members of the insulin signal transduction pathway, including phosphatidylinositol-3-kinase (PI3K) and Akt/protein kinase B (PKB), are recruited and activated in order to induce downstream effects.[58] Insulin signal transduction via PI3 kinase mainly affects metabolic pathways such as GLUT-4 translocation and inhibition of hormone-sensitive lipase. The other main pathway of insulin signal transduction involves signal transduction via the renin-angiotensin system/ mitogen-activated protein (Ras/MAP) kinase pathway and primarily stimulates mitogenic rather than metabolic processes.[58]

Several processes interrupt signalling via the insulin receptor in order to maintain a physiological insulin response. Firstly, protein phosphatases can dephosphorylate the insulin receptor and the IRS proteins; secondly, there may be ligand-induced downregulation of the insulin receptor; and thirdly, insulin receptor signalling induces pathways that inhibit signalling via the insulin receptor. The *physiological* negative feedback mechanism is induced when insulin activates mTOR and PKCζ. These intracellular serine (ser)/threonine (thr) kinases can then either directly, or indirectly (e.g. via IkappaB kinase beta (IKKβ)), phosphorylate ser/thr residues in IRS. Ser/thr phosphorylation of IRS, which occurs at multiple residues in the IRS protein, hampers its tyrosine phosphorylation via insulin receptor and thus interrupts, or at least reduces, insulin signal transduction via the IRS proteins.[59] In addition, ser/thr phosphorylation can induce dissociation of IRS proteins from the insulin receptor,[60,61] induce degradation of IRS proteins,[62,63] remove IRS proteins from complexes that keep them in close proximity to the insulin receptor,[64,65] and turn IRS proteins into inhibitors of insulin receptor kinases.[66]

There are various other intracellular and extracellular substances that can also induce ser/thr phosphorylation of the IRS proteins and thereby hamper insulin signalling, but with *pathophysiological* consequences.[57] These include, for example, the proinflammatory cytokines TNF-α,[67-69] IL-6[70] and IL-1α,[71] and saturated NEFA,[72] which are all involved in obesity-associated low-grade inflammation. These factors employ various intracellular ser/thr kinases such as Jun NH_2-terminal kinase (JNK), protein kinase C (PKC), IKKβ and mTOR,[57,72] which can be activated via multiple mechanisms. IKK-β is particularly interesting in this respect since it is a central effector protein in the inflammatory responses that are activated upon stimulation of the intracellular protein transcription factor NF-κB. Notably, the factors described here mainly affect signal transduction via IRS, and it has indeed been shown that it was the PI3 kinase pathway that was impaired in obesity and in T2D, while insulin signalling via MAP kinase was largely unaffected.[73] Moreover, (saturated) fatty acids, TNF-α and IL-6 have all been demonstrated to induce insulin resistance in healthy humans,[74,75] suggesting that the above-described induction of insulin resistance is indeed relevant in humans, even though a large body of information was obtained in cell and animal studies.

Obese, hypertrophic and/or insulin resistant adipocytes were shown to have an increased release of fatty acids. Specifically, the saturated fatty acids that are released can, in a paracrine fashion, activate the TLR-4/NF-κB pathways on macrophages in adipose tissue, which then release TNF-α, which in turn binds to TNF receptors on the adipocytes, further stimulating fatty acid release and thus inducing a vicious cycle of worsening inflammation and insulin resistance.[76,77] JNK is activated upon exposure not only to cytokines and NEFA, but also to internal cues such as ER stress.[49] Given the relevance of NAFLD in insulin resistance and T2D, it is also of interest that experimental activation of JNK in the liver appeared to be sufficient to induce systemic insulin resistance.[78] The proinflammatory effects of fatty acids appeared to be mainly restricted to the saturated fatty acids while unsaturated and in particularly ω-3 fatty acids, in contrast, appeared to exert anti-inflammatory effects.[79,80]

Inflammation – insulin signalling | Key points:

- In obesity and T2DM it is insulin signalling via IRS and PI3 kinase – i.e. the metabolic pathway – that is primarily affected
- Inflammatory cytokines, e.g. TNF-α and IL-6, as well as saturated fatty acids can all hamper insulin signalling via the IRS and PI3 kinase pathway via activation of intracellular ser/thr kinases
- IKK-β and JNK are important intracellular mediators in metabolic insulin resistance

HOW DOES INFLAMMATION CAUSE β-CELL DYSFUNCTION?

The healthy β-cell has a large capacity to maintain normoglycaemia via an increase in β-cell mass and subsequent hyperinsulinaemia.[81] However, once the demand for insulin exceeds its production, hyperglycaemia will develop (*figure 1*). β-cell failure can result on the one hand from an intrinsic insulin secretion defect in existing β-cells and on the other hand from reduced β-cells mass.[82] β-cell failure may be partly due to genetic and partly to acquired factors. It is probable that genetic disposition may render some individuals more sensitive to those acquired factors than others. Prolonged exposure of pancreatic β-cells to high levels of glucose and lipids, also known as glucotoxicity and lipotoxicity, may contribute to oxidative stress – potentially via effects on mitochondrial function – and to high rates of β-cell apoptosis in T2D.[83-86] Moreover, impaired insulin signalling may add to β-cell dysfunction.[87,88] In addition, inflammatory cytokines may also contribute to β-cell dysfunction and, as such, to enhanced development of T2D.[89]

Hyperglycaemia can induce the production of IL-1β by β-cells,[82,90] and this proinflammatory cytokine was shown to be involved in β-cell deterioration in both T1D and T2D.[91,92] IL-1β may, via induction of specific signal transduction pathways that include Fas (CD95), initially induce β-cell proliferation, but with prolonged hyperglycaemia switch to increased β-cell apoptosis.[93] Notably, leptin, which circulates in considerably increased concentrations in obesity, was shown to increase the release of IL-1β by β-cells.[94] In addition to its effects on β-cells, IL-1β may also induce insulin resistance via direct effects in insulin signalling. For example, IL-1β can down-regulate IRS mRNA expression in adipocytes.[95] The relevance of IL-1β in human T2D, and in particular β-cell function, was recently shown in a placebo-controlled proof-of-concept study with an IL-1 receptor antagonist.[96,97] Clearly, the effects of IL-1β are not the only way through which β-cell mass and function are affected in the development of T2D, but the IL-1β pathway is a relevant representative of the many (inflammatory) pathways that are involved in the generation of β-cell failure in response to obesity-associated low-grade inflammation and the concomitant increased insulin demand.

Pancreatic lipotoxicity partly results from dyslipidaemia (high small dense LDL cholesterol, low HDL cholesterol, high NEFA) and partly from accumulation of fat (triglycerides) in the pancreas as an ectopic fat depot. Increased concentrations of NEFA, particularly saturated fatty acids, were shown to be harmful for β-cells, in among other ways via the induction of IL-1β,[98] and induced an inflammatory response in pancreatic islets.[99] NEFA also induced the local production of other IL-1-dependent proinflammatory cytokines such as IL-6 and IL-8.[99] It was also recently shown that insulin gene transcription was decreased when JNK was activated by palmitic acid in pancreatic β-cells.[100] In addition, reduction of pancreatic triglyceride content was shown to improve insulin secretion capacity.[101]

The effects of lipotoxicity may be enhanced in case of hyperglycaemia.[102] Thus, both glucotoxicity and lipotoxicity induce local production of cytokines and inflammation in pancreatic islets, but it remains to be established to what extent circulating cytokines can also directly affect β-cell survival at their systemic concentrations, although they do appear to affect the secretory function of β-cells, *in vitro*.[103] Other mechanisms that were proposed to explain β-cell failure in obesity-associated T2D include ER stress, oxidative stress and amyloid deposition. Most of these mechanisms have also been implicated in inflammation, either because they induce a (local) inflammatory response or because they result from inflammation.[104] The detrimental effects of inflammation on β-cell function may be particularly relevant in situations of a sustained inflammatory response, as is probably the case in obesity and associated glucose and lipid overload.[104] Increased numbers of macrophages have been shown in pancreatic islets of T2D patients,[99,105] most likely in response to increased islet expression of IL-1β and chemokines.[99,106]

Inflammation and β-cell failure | Key points:

- Glucotoxicity and lipotoxicity may both contribute to β-cell failure, in among other ways via induction of local production of cytokines, e.g. IL-1β, and hence of inflammation in the pancreatic islets
- ER stress, oxidative stress and amyloid deposition may also induce inflammation and β-cell failure in obesity-associated T2D

HOW DOES INFLAMMATION CAUSE MACROVASCULAR DISEASE IN T2D?

CVD comprises the major long-term complication of diabetes. Various aspects of (obesity-associated) inflammation and macrovascular disease have been extensively reviewed elsewhere.[16,107-109] In short, atherosclerosis, the main process underlying macrovascular disease, starts with activation of the endothelial cells that line the intima. Endothelial cell activation, which may be induced by e.g. lipids (including NEFA and cholesterol) or inflammatory cytokines,[16,107-109] can lead to expression of leucocyte adhesion molecules and binding of leucocytes, which migrate through

the endothelium to the intima where they can attract monocytes which ultimately transform into lipid-laden foam cells. These processes may be enhanced in T2D. Further progression of the atheroma and generation of rupture-prone atherosclerotic plaques involves a complex interplay of immune cells and inflammatory mediators. Inflammatory pathways are also involved in thrombosis, the late complication of atherosclerosis which is responsible for most of the complications of macrovascular disease.[107] Macrovascular disease is thus perceived to be a major consequence of obesity-induced inflammation and T2D.

HOW DOES INFLAMMATION CAUSE MICROVASCULAR DYSFUNCTION?

Microvascular dysfunction may not only be a resultant, but also a cause of T2D and hypertension. We recently showed that microvascular dysfunction was associated with a higher incidence of T2D[110] and other studies showed that diet-induced insulin resistance in the microvasculature develops before the development of skeletal muscle insulin resistance.[111,112] How can microvascular dysfunction affect the development of insulin resistance, T2D, and hypertension?

Obese insulin-resistant humans and rats are characterised by impaired capillary recruitment, which has been shown to be necessary for normal insulin-mediated glucose uptake by skeletal muscle.[113] Such microvascular dysfunction may result from increased systemic concentrations of NEFA and inflammatory cytokines, and decreased concentrations of adiponectin, which can induce endothelial insulin resistance, reduce local NO production, lower insulin-mediated glucose uptake in muscle by as much as 40% and, as such, contribute to whole body insulin resistance. Microvascular dysfunction may be further aggravated in the expanding adipose tissue since adipose tissue produces all factors of the RAS necessary to produce angiotensin II, and RAS activity is enhanced in obesity.[113,114] Perivascular fat around resistance arterioles of muscle may directly affect the function of these vessels and indeed it appeared that in lean mice perivascular fat had a beneficial effect to stimulate insulin-induced vasodilation due to local adiponectin production, which was hampered in obese mice.[115] Moreover, this impairment in obese mice was ameliorated by inhibition of JNK.[115]

Microvascular dysfunction may also contribute to the vicious cycle of adipose tissue dysfunction and inflammation. Functional capillaries in the expanding adipose tissue are necessary to provide optimal blood flow and delivery of nutrients and oxygen to adipocytes. Thus, insufficient adipose tissue angiogenesis and capillarisation may lead to hypoxia and induction of an inflammatory response.[116] A relative reduction in the density of the capillary network combined with microvascular dysfunction may therefore aggravate the hypoxic and inflammatory processes in adipose tissue depots and thus lead to deterioration of insulin resistance and metabolic homeostasis.[117]

Microvascular dysfunction may additionally contribute to the development of T2D via effects on β-cell function. For example, transient periods of (mild) hyperglycaemia that coincide with insulin resistance as well as low-grade inflammation – possibly in combination with increased NEFA and dysregulation of adipokines – may lead to reduced islet perfusion and (mild) islet ischaemia,[118] and control the recruitment of inflammatory cells to the islets.[119]

Interestingly, microvascular dysfunction is also thought to contribute to the development of hypertension (reviewed elsewhere[120]), and may thus provide an explanation, at least in part, for the typical co-occurrence of insulin resistance and hypertension in obesity.

Inflammation – vascular disease | Key points:

- Endothelial dysfunction: a shared factor underlying both micro- and macrovascular dysfunction
- Macrovascular disease is a major consequence of obesity-induced inflammation and T2D
- Microvascular dysfunction may be both cause and consequence of obesity-induced inflammation and T2D
- Microvascular endothelial insulin resistance may lead to reduced capillary recruitment in muscle and, as such, contribute to whole body insulin resistance
- Microvascular dysfunction may also contribute to adipose tissue hypoxia and dysfunction

INITIATION OF INFLAMMATION IN OBESITY: RECENT INSIGHTS

Although the concept of low-grade inflammation as an important causal factor in obesity-associated insulin resistance is currently well accepted, less is known about the processes that induce the inflammatory response in adipose tissue. Several processes have been proposed, including the above-described adipocyte hypertrophy, apoptosis and macrophage infiltration, which most likely act simultaneously. Recently, inflammasomes have been proposed as central regulators of early adipose tissue inflammation. Inflammasomes, of which NOD-like receptor family pyrin domain containing 3 (NLRP3) is

the best characterised member, are pattern-recognition receptors (PRRs) that assemble into high-molecular-weight platforms that control maturation and secretion of proinflammatory interleukins such as IL-1β.[121] NLRP3 releases bioactive caspase-1 which can cleave procytokines into their mature active forms.[122] The expression of inflammasome NLRP3 components is increased in obesity, while whole-body knockout of components of this complex resulted in protection from obesity (due to higher energy expenditure), and from inflammation and insulin resistance in mice.[56] Several endogenous stress signals, including glucose, palmitate, cholesterol crystals, islet amyloid peptides and reactive oxygen species, have been suggested as potential *in vivo* inflammasome inducers, but their relevance in the aetiology of human obesity and insulin resistance remains to be elucidated.

A growing body of evidence suggests that cellular components of not only the innate but also the adaptive immune system contribute to adipose tissue dysfunction. The stromal vascular fraction of adipose tissue consists of various types of immune cells, in addition to the macrophage populations discussed earlier. For example, the role of proinflammatory T-cells in obesity-induced T2D has gained significant interest in recent years. Human adipocytes and preadipocytes appear to possess the full machinery to prime inflammation and attract T-cells independently of macrophages.[123] Moreover, subcutaneous adipose tissue of T2D patients has increased presence of not only macrophages, but also of proinflammatory T-cells,[124] infiltration of which preceded the infiltration of macrophages in mice fed a high fat-diet.[124,125] T-cells derived from adipose tissue of obese mice produced more interferon-gamma (IFN)-γ than those from control mice,[126] and hampered preadipocyte-to-adipocyte differentiation.[127] T-cells that are infiltrated in adipose tissue may not only attract macrophages, but also skew their differentiation towards the M1 phenotype. In contrast, induction of T-regulatory cells was beneficial and reduced adipose tissue inflammation and insulin resistance.[128,129] Notably, the anti-inflammatory master switch in adipocyte differentiation, PPAR-γ, was recently identified as major driver of visceral adipose-tissue-resident regulatory T-cells.[130]

Another emerging factor that may underlie, at least part of, the inflammatory response that is seen in insulin resistance and T2D is the gut microbiome. Obese humans and rodents were shown to have higher concentrations of gut-derived endotoxins than non-obese, and these can potentially trigger TLRs in e.g. adipose tissue or on pancreatic β-cells, thus contributing to both insulin resistance and β-cell failure.[131,132] Experimental endotoxaemia can induce adipose tissue inflammation and insulin resistance in lean human subjects.[133] Moreover, portal endotoxaemia may contribute to inflammation in hepatic steatosis and be a relevant risk factor for nonalcoholic steatohepatitis (NASH).[134]

Initiation of obesity-induced inflammation – novel insight | Key points:

- Inflammasomes, of which NLRP3 is the best characterised member, were recently proposed as central regulators of early adipose tissue inflammation
- Pro-inflammatory T-cells may comprise an early inflammatory cellular infiltrate and contribute to cytokine release and attraction of additional inflammatory cells
- Composition of the gut microbiome may contribute to, among other things, endotoxaemia which may induce adipose tissue inflammation

Below we will discuss in more detail two additional emerging early activators of adipose tissue inflammation in obesity: the complement system and advanced glycation end products (AGEs). We will also discuss their potential roles in the development of diabetes.

THE COMPLEMENT SYSTEM IN INFLAMMATION AND T2D

The complement system is a complex protein network that was initially identified as part of the innate immune system. Historically, the liver was regarded the major source of complement, but in recent years, various non-hepatic sources of complement, including adipose tissue and endothelial cells, have been identified. Complement can be activated via several pathways – the classical, the lectin and the alternative – which all converge on complement C3, the central component of the complement system. The alternative pathway also functions as an 'amplification loop' and thereby enhances complement activation once it is initiated by activation of any of the three pathways. All three pathways result in the activation of the terminal complement pathway (*figure 2*). The complement system is increasingly recognised as an essential regulator of cell and tissue homeostasis, in addition to its well-known role in immunity.[135,136] Higher systemic C3 concentrations have been associated with several diabetes risk factors, including obesity, insulin resistance and NAFLD,[137,138] and were shown to be independently associated with incidence of T2D, at least in men.[139]

Various lines of evidence suggest a biologically relevant, functional role for complement activation in adipose tissue homeostasis and insulin resistance. First, adipose tissue expresses a large variety of complement

components and regulators, with contributions from both the stromal vascular and the adipocyte fractions.[140-145] It has been known for over two decades that the alternative complement pathway is activated in adipocytes,[146] and more recently expression of proximal components of the classical pathway has been shown to be altered in subcutaneous adipose cells of insulin resistant individuals.[147] There may also be local effects of complement activation in specific ectopic fat depots. As an example, it was recently reported that the induction of complement C3 from perivascular fat may adversely affect adventitial fibroblast function[148] and complement activation was shown in human NALFD.[149] In addition, C1q, which is an early component of the classical complement pathway, can suppress macrophage inflammation *in vitro,*[150] while, on the other hand, the anaphylatoxins (C3a and C5a) that are generated during complement activation can efficiently attract inflammatory cells to the site of complement activation[151] and, as such, also induce inflammation and insulin resistance in adipose tissue and the liver. C3a-receptor knock-out mice are protected from high-fat-diet-induced insulin resistance and have less macrophage infiltration in adipose tissue[152] and, very recently, similar data were obtained in C5a-receptor knock-out mice.[153] In particular the observations on C3a and C5a and their receptors suggest that complement activation in obesity, and in particular in hypertrophic, metabolically stressed adipocytes, may contribute to recruitment of immune cells from the circulation to the adipose tissue. This may enhance the cellular immune response in adipose tissue and thereby contribute to adipose tissue dysfunction.

In addition to the proposed effects of complement activation on adipose tissue (dys)function, there may also be a role for complement activation in the progression of β-cell dysfunction in T2D, as absence of complement C3 in mice prevents diabetes development in response in mice treated with low-dose streptozotocin,[154,155] which causes β-cell deterioration in a way similar to what is observed in T2D.[82] There is also a substantial body of data, including from our own research group,[156] that implicates complement activation in the development of macrovascular and microvascular disease[156-158] (*figure 2*) as reviewed elsewhere.[135,137,159] In short, complement system activation leads to endothelial dysfunction, especially when protection of the endothelial cells is compromised due to decreased expression of complement inhibitors.[160] Since the microvasculature comprises approximately 98% of the total vascular surface area,[161] complement activation will inevitably affect microvascular function. With respect to atherosclerosis, complement activation by the classical and perhaps also the lectin pathway may aid in removal of apoptotic cell and cell debris and hence have protective effects in the atherosclerotic plaque, while complement activation beyond C3, which is associated with the release of anaphylatoxins and assembly of the (soluble) terminal complex, may be proatherogenic.[159] Moreover, complement activation may be instrumental in the development of atherothrombosis since proteases of the coagulation and fibrinolysis systems may activate the complement system, and vice versa.[162]

Complement – inflammation – T2D | Key points:

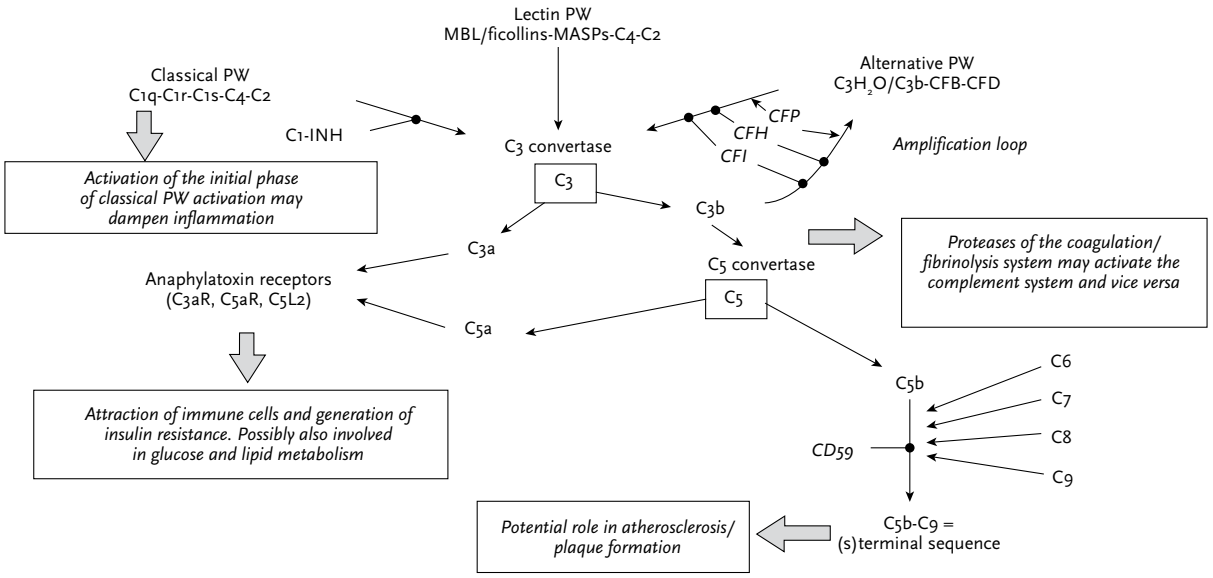
- Many complement components are produced by human adipose tissue (by both adipocytes and stromal vascular cells), and are increased in obesity, insulin resistance and low-grade inflammation
- Complement activation in adipose tissue, liver or pancreatic islets may contribute to inflammation and attraction of immune cells
- Complement activation may lead to endothelial dysfunction, and has been implicated in macro- and microvascular disease

Advanced glycation, inflammation and T2D

AGEs form a heterogeneous family of unavoidable by-products that are formed by reactive metabolic intermediates derived from glucose and lipid oxidation.[163] In addition to the overwhelming amount of data, including ours,[164-167] demonstrating a role of AGEs in the development of vascular disease in diabetes (reviewed elsewhere[168,169], AGEs are implicated in the development of obesity and diabetes[170] and have been found to be associated with insulin resistance.[171] In obesity, the combined effects of enhanced food consumption, low energy expenditure, hyperglycaemia, hyperlipidaemia and increased oxidative stress may augment the formation of specific AGEs such as N[Carboxymethyl)lysine (CML). Peroxidation of lipids may also lead to the formation of the reactive dicarbonyl compound methylglyoxal (MGO), which is believed to be the most potent glycation product. Accelerated endogenous formation of both CML and MGO in obesity has been described in a few studies. We recently demonstrated the accumulation of a major AGE, CML, in adipose tissue and fatty liver and provided evidence that this is a core mechanism leading to the dysregulation of cytokines production.[172,173] CML is a major ligand for the receptor for AGE (RAGE) and we demonstrated that $RAGE^{-/-}$ $obese^{db/db}$ mice have reduced inflammation and improved insulin sensitivity, indicating a role for the CML-RAGE axis in inducing insulin resistance.[173]

In addition to the effects in insulin resistance, AGEs have also been shown to induce β-cell dysfunction and apoptosis, at least partly via the AGE-RAGE axis[174-177] and via effect of MGO.[178,179]

Figure 2. *Complement activation and the development of vascular disease*



Many complement components are expressed in adipose tissue, by adipocytes, (resident) immune cells, or both. Complement can be activated via three pathways (PW), the classical, the lectin and the alternative PW. C1q, the first component of the classical PW, is activated by antibodies and by members of the pentraxin family (which includes e.g. C-reactive protein (CRP)) resulting in formation of the C1qrs complex that subsequently activates complement factors C4 and C2. C1 inhibitor (C-INH) inhibits C1R and C1S and is a major component for controlling the classical PW; the lectin PW is activated via mannose-binding lectin (MBL) or ficolins which subsequently bind and activate MASPs (MBL-associated serum proteases), which in turn activate C4 and C2. These last-mentioned complement components are shared between the lectin and the classical PWs. The alternative PW has a slow intrinsic auto-activation (via $C3H_2O$), but also functions as an important 'amplification loop' and thereby enhances complement activation once it is initiated by activation of any of the three pathways. Activation of the alternative pathway results in activation of factors B and D. Alternative PW amplification (and hence the amplification loop) is controlled by properdin (CFP, positive regulator) and factors I and H (CFI/CFH, negative regulators). All complement PWs result in the generation of C3 convertases which are able to activate C3; C3 activation in turn initiates the formation of C5 convertases that can activate C5. Activation of C3 and subsequently C5 generates C3b and C5b, respectively, with the release of the anaphylatoxins C3a and C5a, which can activate one or more surface receptors.C5b assembles with C6 through C9 to form the (sublytic) terminal complement sequence (C5b-9) on cells. This last step of complement activation is controlled (inhibited) by CD59. Complement activation is under tight control of a large number of circulating and cell-bound inhibitors to prevent unrestrained progression of complement activation once any of the three pathways is initiated and only a few of those are included in this simplified scheme. Complement activation may be involved in the development of cardiovascular disease via several mechanisms and at several levels, as indicated

Advanced glycosylation end products – inflammation – T2D | Key points:

- Obesity is characterised by increased formation of advanced glycation end products
- AGEs in obesity may have important biological effects on the dysregulation of adipokine secretion and the induction of insulin resistance
- AGEs have been shown to induce β-cell dysfunction

CONCLUDING REMARKS

Taken together, the larger picture on how obesity, inflammation and T2D are interrelated is becoming increasingly clear. We have provided an overview of the different fat depots and their potential contribution to obesity-associated inflammation, on how inflammatory cytokines can affect insulin signalling at the molecular level and on how similar molecular events may also affect β-cell function. We have additionally discussed novel insights into the processes that may initiate the obesity-associated inflammatory response, including complement activation and advanced glycation end products. However, the details on exactly where and how inflammation is induced, the temporal order of the events that contribute to insulin resistance and the development of β-cell function, and the role of vascular dysfunction therein remain to be further elucidated. More detailed knowledge of these events will help to pin-point optimal targets for prevention of, and intervention in, T2D.

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REVIEW

Established and forthcoming drugs for the treatment of osteoporosis

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ABSTRACT

Nowadays, effective drugs are available to prevent fractures in patients at high risk for osteoporotic fractures. The generic bisphosphonates alendronate and risedronate are first choice, because of their effectiveness and tolerability in the majority of patients, while they also have a low cost price. However, the use of bisphosphonates can be associated with side effects: not only the well-known (upper) gastrointestinal side effects, but also (spontaneous) atypical fractures of the femur and aseptic necrosis of the jaw.

Denosumab and zoledronic acid are both potent antiresorptive drugs that could be an attractive alternative for those patients who do not tolerate oral bisphosphonates. Strontium ranelate has both antiresorptive and anabolic effects, while teriparatide has primarily anabolic effects. The working mechanism of cathepsin K inhibitors and monoclonal antibodies against sclerostin, both currently under development, is exciting since the usually occurring coupling of bone resorption and bone formation has not been found so far.

KEYWORDS

Osteoporosis, bisphosphonates, denosumab, cathepsin K inhibitor

INTRODUCTION

Osteoporosis is a skeletal disease that is characterised by low bone mineral density (BMD) and a microarchitectural deterioration of bone tissue, leading to decreased bone quality and an elevated risk of fractures.[1] Patients 50 years and over with a recent clinical fracture and a low BMD, and elderly patients with (several) clinical risk factors for osteoporosis, such as low BMI, familial osteoporosis and immobility, and a T-score below -2.5 at the lumbar spine and/or hips are usually prescribed antiosteoporotic treatment.[2] In addition, elderly osteopenic patients (T-score between -1 and -2.5) who are on chronic treatment with prednisone (>7.5 mg per day) or elderly osteopenic patients with one or more vertebral fractures, are also offered antiosteoporotic treatment.[3] General measures are necessary in all osteoporotic patients: adequate calcium and vitamin D intake, regular (weight-bearing) exercises, prevention of falls, smoking cessation and avoiding (a large intake of) alcohol.[2]

In this manuscript we will discuss the effectiveness and side effects of the antiosteoporotic drugs calcium and vitamin D supplements, oral bisphosphonates, strontium ranelate, raloxifen, zoledronic acid, and also new drugs with exciting working mechanisms that either have a stimulating effect on bone formation (teriparatide, PTH 1-84), selectively block RANKl (denosumab), or are currently under development (cathepsin K inhibitors, monoclonal antibodies against sclerostin).

CALCIUM AND VITAMIN D

Vitamin D plays a pivotal role in the uptake of calcium and also has direct effects on bone. Vitamin D and calcium deficiency leads to secondary hyperparathyroidism and osteoporosis and osteomalacia.[4] Therefore, adequate vitamin D and calcium status is the very basis of osteoporosis prevention and treatment. This is illustrated by the fact that almost all trials on antiosteoporotic drugs use vitamin D and calcium supplementation in both the active and the placebo arm. Much debate exists on which levels of 25-hydroxy vitamin D are necessary with regard to the prevention of bone loss. In the Netherlands, the 2008 report of the Health Council advised a level of 50 nmol/l as sufficient.[5] Recently,

the Institute of Medicine declared the skeletal effects of vitamin D as the only proven effect and also recommended a level above 50 nmol/l.[6] Based on these levels patients with osteoporosis are advised to take 800 IE vitamin D daily.[2,5] Patients with coeliac disease and other bowel disorders with intestinal malabsorption should be tested whether they achieve these levels. Furthermore, patients with chronic kidney disease should receive calcitriol or another active vitamin D because of low 1-alpha hydroxylase activity.

The recommended daily allowance (RDA) for calcium in adults is 1000-1200 mg.[6] Some concern was raised after the publication of a meta-analysis that showed an increased risk for cardiovascular disease in subjects treated with higher doses of calcium alone (without vitamin D).[7] However, a number of issues with the studies, such as inadequate compliance with the intervention, use of non-trial calcium supplements, potential bias in event ascertainment, and lack of information on and adjustment for known cardiovascular risk determinants, suggest that bias and confounding cannot be excluded as explanations for the reported associations.[8] Findings from other cohort studies also suggest no detrimental effect of calcium from diet or supplements, with or without vitamin D, on cardiovascular disease risk. So the current RDA of 1000-1200 mg still holds.[2]

BISPHOSPHONATES

Where lifestyle recommendations such as weight-bearing exercises, fall prevention, smoking cessation and the use of sufficient calcium and vitamin D is essential to all osteoporosis prevention, in patients with documented osteoporosis this will not be sufficient. Bisphosphonates have been cornerstone in osteoporosis treatment for a long time. The first trials were done in the 1970s. Bisphosphonates have a structure that resembles hydroxyapatite and are built into the bone. They are toxic for the osteoclasts by interfering with adenosine triphosphate (ATP) in the energy metabolism (non-nitrogenous bisphosphonates) or with the HMG-CoA reductase system (nitrogenous bisphosphonates) and therefore act primarily as antiresorptive agents. More than ten different bisphosphonates are available for both oral and intravenous use.

Etidronate is one of the first bisphosphonates used in clinical practice, but its antiresorptive potency is low compared with more recently developed nitrogenous bisphosphonates (such as alendronate, risedronate, and zoledronic acid). Because etidronate at higher doses can induce mineralisation defects (osteomalacia), the nitrogenous bisphosphonates are now recommended. Because of the data showing a reduction in vertebral and nonvertebral fractures, including hip fractures, the long-term experience, the tolerability in the majority of patients and the low cost price of the generics, alendronate and risedronate are first choice.[2]

Other bisphosphonates, e.g. ibandronate, tiludronate and pamidronate, also reduce bone resorption, but fracture reduction (vertebral, nonvertebral and hip fracture) has not been documented.

The main problem in oral therapy is to achieve the desired levels in the blood. For most patients the oral form will be sufficient, but in cases of gastrointestinal symptoms or gastrointestinal contraindications, the intravenous form could be chosen. Although several intravenous bisphosphonates are available, zoledronic acid has been most extensively studied and showed a strong reduction in both vertebral and nonvertebral fractures.[9]

One of the side effects of oral bisphosphonates is the damage to the oesophageal mucosa which can result in ulcers and eventually in oesophageal cancer. Therefore patients should be told to take these pills in a fasting state and to remain in an upright position for half an hour. Given these recommendations bisphosphonate use turned out not to be associated with a higher incidence of oesophageal cancer.[10]

In the last decade two other side effects became apparent, osteonecrosis of the jaw (ONJ) and atypical fractures.[11] Both are thought to result from the low bone turnover state that is induced by the bisphosphonates. Although the primary effect is antiresorptive, the close connection between osteoclasts and osteoblasts results not only in decreased bone resorption but also decreased bone formation in the long term.

Osteonecrosis of the jaw occurs more often in patients with periodontitis. Therefore doctors prescribing bisphosphonates should ask about dental history and dentists should ask about bisphosphonate use in their patients. It is important to realise that the risk of ONJ is much higher in patients with a malignancy and who are treated with several courses of intravenous bisphosphonates than in postmenopausal osteoporotic women treated with oral bisphosphonates.

Atypical subtrochanteric hip fractures are a relatively recently discovered side effect of long-term bisphosphonate treatment. It is a fracture in the subtrochanteric or shaft region with a transverse or short oblique orientation and with a thickening of the cortex. This type of fracture is most often reported in combination with long-term (>5 years) bisphosphonate use. Although these side effects of bisphosphonates may be serious, the incidence is relatively low. However, especially the occurrence of atypical fractures in long-term bisphosphonate use has warranted a more restricted use and the introduction of a 'drug holiday' of two to three years after the initial treatment for five years.

Most trials with bisphosphonates excluded patients with chronic kidney disease (CDK), especially with an estimated creatinine clearance below 30 ml/min. Next to the paucity of data an important reason to withhold bisphosphonates in CKD patients is the occurrence of other bone diseases that result in a lower bone density (for instance renal osteodystrophy).[12]

STRONTIUM RANELATE

Strontium ranelate is a unique drug, since it has a combined effect with stimulation of bone formation and inhibition of bone resorption. Strontium is an elementary element such as calcium and is named after the Scottish place, Strontian, were this element was found.[13] It has been used for osteoporosis for a long time. Strontium ranelate acts on the surface of the bone and stimulates the differentiation of osteoblasts by stimulating the calcium sensor receptor, but inhibits osteoclast differentiation by inhibiting RANKL production and increasing osteoprotegerin (OPG) activity.

Two phase III trials show a clear effect on the prevention of vertebral fractures: the Spinal Osteoporosis Therapeutic Intervention study (SOTI) with 1649 postmenopausal women with osteoporosis and at least one vertebral fracture showed a 41% reduction in new vertebral fractures after three years.[14] The TReatment Of Peripheral OSteoporosis study (TROPOS) with 5091 postmenopausal women with osteoporosis also showed, in three years, a 16% reduction in nonvertebral fractures and a 36% reduction in hip fractures in patients with high risk of hip fracture.[15] The medication was well tolerated, and the safety profile was similar to that in younger patients. Although very rare, strontium ranelate can induce DRESS (drug rash with eosinophilia and systemic symptoms). Recently, it has been demonstrated that strontium ranelate reduces radiological progression and pain in patients with knee osteoarthritis. This is important, because osteoporosis and osteoarthritis often coexist in the elderly, and only symptomatic drugs are available in osteoarthritis.[16]

RALOXIFENE

Raloxifene is a selective oestrogen receptor modulator that acts as an oestrogen agonist for bone, lipids and the coagulation system, and as an oestrogen antagonist on breast and uterus. It has a preventive effect on vertebral fractures, but not on hip fractures.[17,18] Additionally it has a protective effect on breast cancer. However, as with oestrogen use, it is associated with an increased risk for venous thrombosis. Raloxifene is not very often prescribed for the prevention of osteoporotic fractures because it has only been proven that it reduces vertebral fractures and because of its side effects. It might be an attractive antiosteoporotic drug in relatively young women (around 60-65 years of age) with oestrogen receptor positive breast cancer.

PTH

Nowadays, two parathyroid hormone (PTH) analogues are available for the treatment of established osteoporosis in postmenopausal women (PTH 1-34 and PTH 1-84).

These PTH analogues are unique anabolic drugs since they not only more or less preserve the BMD by blocking osteoclast activity (such as bisphosphonates), they also build up new bone, by increasing the number of osteoblasts, resulting in an increase in the percent bone-forming surfaces with little effect on bone resorption.[19] Recent data have shown that intermittent PTH analogues interfere with the WnT signalling pathway, by blocking sclerostin, leading to stimulated bone formation.[20]

In the Fracture Prevention Trial (FPT), a multicentre, randomised, double-blind, placebo-controlled trial, 1637 postmenopausal women with an average age of 69 years, an average T-score of -2.6 and at least one prevalent osteoporotic vertebral fracture at the beginning of the study, were included.[21] New vertebral fractures occurred in 14% of the women in the placebo group and in 5% and 4%, respectively, of the women in the 20 μg and 40 μg PTH 1-34 groups; the respective relative risks, as compared with the placebo group, were 0.35 and 0.31 (95% CI intervals 0.22 to 0.55 and 0.19 to 0.50). New nonvertebral fragility fractures occurred in 6% of the women in the placebo group and in 3% of those in each PTH 1-34 group: relative risk 0.47 and 0.46, respectively (95% CI 0.25 to 0.88 and 0.25 to 0.861). No reduction in hip fractures was observed, probably related to the low number of hip fractures in the study (four hip fractures in the whole study).

In the TOP (Treatment of Osteoporosis with Parathyroid Hormone) study with PTH 1-84, a multicentre, randomised, double-blind, placebo-controlled clinical trial on 2532 postmenopausal women either with or without prevalent osteoporotic vertebral fractures, a relative risk reduction of new vertebral fractures was found: 0.42 (95% CI 0.24 to 0.72), but no reduction in nonvertebral and/or hip fractures.[22] The key question is whether PTH 1-84 is less effective than PTH 1-34, or that the design of the study plays an important role, e.g. the enrolment of patients with a lower baseline fracture risk.

Recently, the EFOS (European Forsteo Observation Study) data were published, a large observational study in 1648 patients in eight European countries, all treated for 18 months with teriparatide.[23] In general, these patients had

severe osteoporosis, and several of them had co-morbidities and/or used co-medication that made them not eligible for the above-mentioned phase III trials. The main outcome of the study was a substantial decrease in fracture rate over time: a 47% decrease in fracture rate in the last six-month period compared with the first six-month period. Moreover, a substantial decrease in back pain was found: visual analogue scale at baseline was 57.7 mm, and this was reduced by 25.8 mm after 18 months of treatment ($p<0.001$). In addition to that, an increase in quality of life was observed.

Given the fact that glucocorticoids (GC) induce apoptosis of osteoblasts and osteocytes, with a subsequent inhibiting effect on bone formation, an anabolic drug would, at least theoretically, be preferred to a bisphosphonate for the treatment of glucocorticoid-induced osteoporosis (GIOP).[24] In 2007, PTH 1-34 (20 μg/day) was compared with the active comparator alendronate (10 mg/day) for treating GC-induced osteoporosis in a 36-month, randomised, double-blind, controlled trial in 428 subjects with osteoporosis who had received prednisone 5 mg/day or more for at least three months.[25] A reduction in vertebral fractures in the PTH 1-34 group versus the alendronate group was observed: (3 [1.7%] versus 13 [7.7%]; $p = 0.007$), but no significant difference in the incidence of non-vertebral fractures. In an editorial by Phil Sambrook it was concluded that for patients with low bone mineral density who are receiving long-term GC therapy, teriparatide should be considered as a potential first-line therapy.[26] However, at least partly related to the high cost price, reimbursement of teriparatide (and PTH 1-84) is limited: e.g. in the Netherlands it is only reimbursed for postmenopausal women, and since 1 March 2013 also for men, with two prevalent vertebral fractures who suffer from a third fracture during treatment with bisphosphonates or strontium ranelate.[19]

DENOSUMAB

Denosumab is a really new approach to the prevention of osteoporotic fractures. It is a fully human monoclonal antibody that binds to RANKl and inhibits the RANKl-RANK interaction, resulting in suppressed formation, function and survival of osteoclasts.[27] During treatment with denosumab, bone resorption is strongly inhibited, as shown by a strong reduction in serum C-telopeptide, a marker of bone resorption, one month and six months after subcutaneous injection: -86% and -72%, respectively.[28] In the randomised, pivotal phase III trial, 7868 postmenopausal women with a T-score between -2.5 and -4 were enrolled, and were treated with subcutaneous injections of 60 mg of denosumab or placebo every six months for three years. Remarkably, only 24% of the patients had vertebral fractures at baseline, indicating that fracture risk was relatively low in this patient group. Nevertheless, a striking reduction in the number of patients with new vertebral fractures, the primary endpoint, was found: relative risk 0.32, 95% CI 0.24 to 0.41. In an accompanying editorial is was suggested that a difference was observed between moderate reductions of vertebral fractures for oral bisphosphonates and strontium ranelate, and much larger reductions, for zoledronic acid and denosumab.[29] In addition, a risk reduction of 40% was found for hip fractures and (only) 20% for nonvertebral fractures.

No significant difference was found in serious infections or malignancies; eczema and cellulitis were more frequently reported. As far as we know, no phase IV studies are currently underway in the Netherlands observing the side effects of denosumab in clinical practice in osteoporotic patients with co-morbidity and/or co-medication.

In a follow-up study, patients were eligible to enter a two-year extension study: thus, patients were treated with five years of denosumab, or with two years of denosumab, after three years with placebo.[30] In the five-year denosumab group, BMD increased at the lumbar spine to 13.7%, and to 7.0% at the hips; in the crossover group to 7.7% and 4.0% respectively. Apart from this large increase in BMD, the yearly incidence of both vertebral and nonvertebral fractures were comparable with the relatively low fracture incidence in the denosumab group in the first three years of the study (despite ageing).

In human rheumatoid arthritis (RA) patients, twice-yearly subcutaneous injections of 60 mg and 180 mg of denosumab to RA patients treated with methotrexate not only improved BMD of the lumbar spine and hip but also inhibited structural joint damage.[31] Both dosages prevented progression of erosions, already after six months on MRI and after 12 months on conventional X-rays. In addition, an increased BMD of the hand measured with DXA was observed, while no effect was observed on joint space narrowing. These data indicate that both local and generalised bone loss in RA can be protected by using potent antiresorptive therapies specifically targeting osteoclasts without effecting inflammation.

Cathepsin K inhibitors

The structure and the physiological role of cathepsin K was discovered in 1995; it was found that cathepsin K is the protease that primarily induces the degradation of bone matrix by osteoclasts.[32] Since then, several cathepsin K inhibitors have been developed, while the development was blocked in one of them because of serious side effects (scleroderma-like changes in the skin). For odanacatib, a selective cathepsin K-inhibitor, reliable data are available from phase II studies, and the phase III study is nearly finished. In a randomised controlled trial in 339 patients,

odanacatib 50 mg once weekly was compared with placebo: a greater increase in the BMD of both the lumbar spine and the total hip was observed: 5.5% versus -0.2% and 3.2% versus -0.9%, respectively.[33] Although this increase is impressive, and probably larger than for oral bisphosphonates, it is not yet clear how to extrapolate this to (additional) fracture reduction. During treatment with odanacatib, one very exciting phenomenon occurs: while bone resorption and bone formation are usually coupled, there seems to be some uncoupling in odanacatib-treated patients. For example, serum C-terminal telopeptide (CTX) decreased during treatment with odanacatib, more or less comparable with antiresorptive drugs, while markers of bone formation only initially decrease but then gradually return back to baseline. It is thought that cathepsin K targets the resorption process itself, without an osteoclast-medicated effect on bone formation. Nowadays, we can only speculate whether the absence of uncoupling is not only scientifically and theoretically attractive, but also clinically relevant. A phase III trial with hip fractures as one of the endpoints was terminated in July 2012 because of robust efficacy data; the final results are expected in 2013.

MONOCLONAL ANTIBODIES AGAINST SCLEROSTIN

Sclerosteosis and Van Buchem disease are two closely related rare disorders resulting from endosteal hyperostosis, which are characterised by progressive generalised osteosclerosis, particularly in the mandible and skull, sometimes complicated by entrapment of cranial nerves.[34] Sclerosteosis is caused by a genetic defect on chromosome 17q12-21, encoding for the protein sclerostin; in Van Buchem disease a modification downstream of the same gene was found. In healthy adults, sclerostin is expressed by osteocytes, but not in patients with sclerosteosis and Van Buchem disease sclerostin has an inhibiting effect on bone formation by antagonising the Wnt-signalling pathway. Recently, it has been elucidated that the WnT-signalling pathway plays an important role in bone formation.[34] It is a complex system that can be inhibited by sclerostin, produced by osteocytes, and by Dkk-1. Dkk-1 is upregulated in patients with active RA and in patients treated with glucocorticoids, leading to inhibited bone formation. Theoretically, it is very attractive to develop a strategy to block sclerostin, e.g. by monoclonal antibodies. In a recently published dose-escalating single-dose phase 1 trial it was demonstrated that one single (subcutaneous) injection of a monoclonal antibody against sclerostin markedly increased bone formation markers and BMD: an increase in BMD of 5.3% at the lumbar spine and 2.8% at the total hip was found 85 days after one single injection of 10 mg/kg![35] These data are promising, and the very strong bone formation stimulating effect may be very helpful in patients with severe osteoporosis, although the optimal regimen and dosage should be further investigated.

SUMMARY

Nowadays, the oral bisphosphonates alendronate and risedronate are first choice in the treatment of osteoporosis, because of their effectiveness and tolerability in the majority of patients, while they also have a low cost price. However, the use of bisphosphonates can be associated with side effects: not only the well-known upper gastrointestinal side effects, but also (spontaneous) atypical fractures of the femur and aseptic necrosis of the jaw. The intravenous use of zoledronic acid might be an attractive alternative for those patients who do not tolerate oral bisphosphonates due to gastrointestinal side effects of gastrointestinal contraindications. Strontium ranelate has, next to an antiresorptive effects, also anabolic effects and might particularly be interesting in the elderly, because of the hip fracture reduction in those individuals above 74 years. In addition, it has been demonstrated that strontium ranelate reduces radiological progression and pain in patients with knee osteoarthritis.

Several new drugs are already available or are in the pipeline: these drugs have a completely different mode of action, and seem to induce large changes in BMD. For teriparatide and denosumab substantial decreases in fracture rate have already been documented; both drugs can be prescribed in daily practice. Denosumab is an elegant way of treating osteoporosis since it has the advantage that the favourable effect (vertebral and nonvertebral, including hip fracture reduction) is possible with only two subcutaneous injections per year. Teriparatide is the only drug currently available that has a strong anabolic effect: it leads not only to fracture reduction, but also to reduction in back pain and increased quality of life in elderly postmenopausal women with severe osteoporosis.

For the cathepsin-K antagonist odanacatib fracture data are not (yet) available; for monoclonal antibodies against sclerostin it will probably take some years before phase III trials are finished. Of course, an eventual favourable fracture rate has to be outweighed against data on side effects, cost price of the drugs, and comparisons with already available drugs, such as oral bisphosphonates.

Thus, the number of antiosteoporotic drugs in the field of osteoporosis is rising, and it can be expected that in the coming years very attractive new treatment options can be used in daily practice. Physicians who treat patients with osteoporosis should be aware of the effectiveness,

possible side effects and cost price of these drugs. Apart from that, nonmedical treatment is also still important: adequate calcium and vitamin D supply, regular exercises, prevention of falls, smoking cessation and limiting alcohol intake.

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ORIGINAL ARTICLE

INR control calculation: comparison of Dutch and international methods

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ABSTRACT

Results of trials with new oral anticoagulant drugs and vitamin K antagonists (VKA) might not be directly applicable to Dutch clinical practice due to the high level of control of anticoagulation in the Netherlands. In addition, the Dutch method for assessing anticoagulation control uses cross-sectional international normalised ratio (INR) test results while the method used in the trials is based on person-time.

To enable comparisons, the two calculation methods were applied to INR data of a cohort of 5422 atrial fibrillation patients treated with VKA.

Overall, 74% of test results and 77% of person-time were in the therapeutic range [2.0-3.5]. For the narrower target INR interval [2.5-3.5], 59% of test results and 61% of person-time were in range. It was only between two and six months after the start of treatment that the percentage of person-time in range was lower than the percentage of test results in range. Control of anticoagulation, expressed as a percentage of person-time spent in range, in this Dutch dataset was similar to recent trials with new oral anticoagulants, although it should be noted that the Dutch INR target is higher than the target in these trials. INR control as estimated by the two calculation methods (cross-sectional and longitudinal) was similar.

KEYWORDS

Anticoagulants, atrial fibrillation, INR, TTR, vitamin K antagonists

INTRODUCTION

With the introduction of dabigatran and rivaroxaban, new oral anticoagulant drugs became available in the Netherlands. These drugs are indicated for the prevention of thromboembolic disease following knee or hip replacement, based on comparative studies with low-molecular-weight heparin.[1] More recently, results were published from randomised trials comparing dabigatran, rivaroxaban or apixaban with warfarin (a vitamin K antagonist, VKA) for prevention of cerebrovascular accidents in patients with atrial fibrillation. These studies showed superiority or noninferiority with regard to reductions in stroke or systemic embolism rates and bleeding, compared with warfarin.[2-5]

Effectiveness and safety of VKA treatment depends, among other things, on the intensity of anticoagulation.[6] During VKA treatment, the clotting tendency of the patient's blood, expressed as international normalised ratio (INR), is monitored and VKA doses adjusted if necessary in order to achieve INR values within a specified therapeutic or target range. Different methods exist to assess the level of INR control: a cross-sectional method based on the proportion of INR test results in range[7] and a longitudinal method based on the proportion of person-time spent in range (time in therapeutic range, TTR).[8] The Dutch Thrombosis Service represents a unique, high-standard setting of care for monitoring and dosing of VKA treatment.[7] The reported percentage of cross-sectional INR test results in the therapeutic range is 70-80%.[9-11] The trials that compare the new oral anticoagulants with VKA treatment use the longitudinal method, which hampers extrapolation of internationally obtained results to the Dutch setting. When the level of INR control in the Netherlands differs from the trial settings, the results are not directly applicable. In addition, the therapeutic range in the Netherlands (INR 2.0-3.5) differs from the therapeutic range used in the trials (INR 2.0-3.0).

The aim of this study was to describe how the Dutch cross-sectional INR calculation and the longitudinal Rosendaal method (TTR) compare in order to enable comparisons of the Dutch setting with international studies.

MATERIAL AND METHODS

Data source

The PHARMO Institute was granted access to the data of the Dutch Thrombosis Service in the region of Eindhoven concerning clients using VKA and requiring regular monitoring of INR. For this study the data from 2007 to 2009 were analysed. Variables in the database included indication of type of VKA use, dosing schemes and INR measurements.

Patient selection

All VKA users with an indication of atrial fibrillation who attended the Thrombosis Service Region Eindhoven between 2007 and 2009 (study period) were eligible for inclusion in the study. Study patients started treatment in the study period, or before but continued attending the Thrombosis Service during the study period. Start of treatment was defined as the date of the first INR measurement after the date of registration at the Thrombosis Service. In order to obtain stable estimates of INR control, INR measurements performed within two months of the start of treatment were excluded. Treatment was defined as subsequent INR measurements during use of one specific VKA (acenocoumarol or phenprocoumon). A maximum gap of 12 weeks was allowed between measurements; if the gap was larger, treatment was assumed to be ended. Only the first treatment within the study period was included. Start of follow-up for the study was defined as the date of the first INR measurement in the study period, or the first measurement that was performed after at least two months of treatment for patients starting treatment in or just before start of the study period. Consequently, patients who had received less than two months of treatment were excluded. End of follow-up was defined as the date of the last measurement recorded at the Thrombosis Service, the last measurement under treatment with the specific VKA (switching of therapy), or the last measurement in 2009 (end of study period), whichever came first.

Study endpoints

The percentage of INR measurements within the therapeutic range and the percentage of person-time in the therapeutic range were calculated. The therapeutic and target ranges for atrial fibrillation as defined by the Federation of Dutch Thrombosis Services (the FNT; INR 2.0-3.5 and INR 2.5-3.5)[8-11] were used. Analysis of INR range 2.0-3.0 was not considered useful as the Thrombosis Service is not aiming at that range.

Data analysis

This study was a descriptive analysis of INR test results. Two calculation methods were applied: the percentage of INR measurements within therapeutic or target range using cross-sectional data and the percentage of time in therapeutic or target range based on longitudinal data.

INR test results within range (cross-sectional method)

The percentage of INR test results within range was calculated as described by Van Geest-Daalderop,[7] a method which is also used in the annual reports of the Dutch Thrombosis Service. The Thrombosis Service assesses overall treatment intensity twice a year, by taking the last INR of each patient before the prespecified assessment date and calculating the percentage of INR results within the therapeutic range.[7] In the current study, the data delivery dates of the Thrombosis Service Region Eindhoven are adopted: 31 March and 30 September. In each year during the study period, the last INR of each patient since the last data delivery date (and after at least two months of treatment) was included. The percentage of INR results within range was based on the mean of the included estimates (two per calendar year, in total a maximum of six).

Person-time in range (TTR, longitudinal method)

The percentage of total person-time spent within the therapeutic or target range was calculated as described by Rosendaal.[8] This method 'allocates the person-time between two measurements to particular INR values [...] by dividing the time between two measurements in days, and using small steps of 0.1 INR over the range of the time interval. [...].' In the current study all INR test results during follow-up were included; person-time was allocated to the therapeutic or target range according to the Rosendaal method and summed over all patients.

Stratified analysis

The calculation methods were performed overall and in the following strata: age at start of follow-up (<60, 60-69, 70-79, ≥80 years), VKA (acenocoumarol, phenprocoumon), time between start of treatment and INR measurements (2-6 months, >6 months) and calendar year of INR measurement (2007, 2008, 2009).

Statistical analysis

Data were analysed using SAS programs that are organised within SAS Enterprise Guide version 4.2 (SAS Institute Inc., Cary, NC, USA). Data management was conducted under Windows using SAS version 9.2.

RESULTS

In 2007-2009, 5921 AF patients were treated with VKA of whom 499 patients (9%) had received less than two months of treatment, which resulted in a study cohort of 5422 AF patients (see *table 1* for patient characteristics).

Table 1. *Characteristics of patients treated with VKA for atrial fibrillation*

	Study population n=5422
Gender, n (%)	
Men	2889 (53)
Women	2533 (47)
Age at start of follow-up	
<60	390 (7)
60-69	1013 (19)
70-79	1974 (36)
≥80	2045 (38)
Mean (± SD)	75 ± 10
Vitamin K antagonist, n (%)	
Acenocoumarol	4687 (86)
Phenprocoumon	735 (14)
Start of treatment, n (%)	
<2001	584 (11)
2001-2003	808 (15)
2004-2006	1744 (32)
2007-2009	2286 (42)
Follow-up in months	
0-12	1686 (31)
>12-24	1089 (20)
> 24-36	2647 (49)
Mean (± SD)	22 ± 13
End of follow-up, n (%)	
Last measurement recorded	1629 (30)
Switching of therapy	0 (0)
End of study period	3793 (70)
Number of INR measurements during follow-up, mean (± SD)	41 ± 25
Time between measurements in days, mean (± SD)	17 ± 5

SD = standard deviation.

Table 2. *Percentages of test results (cross-sectional) and person-time (longitudinal) in range*

	Cross-sectional method	Longitudinal method
	INR test results within range n (%)*	Person years within range n (%)
Total	12,064 (100)	9742 (100)
INR 2.0-3.5 (therapeutic range)	8963 (74)	7487 (77)
INR 2.5-3.5 (target range)	7166 (59)	5915 (61)

***One measurement per patient per six months.**

The study population included slightly more men (53%) than women (47%). Mean age was 75 (± 10) years. Acenocoumarol was the primary VKA used in this population (86%); none of the patients switched preparations during the study period. Most patients started treatment in or just before the study period; 26% had been on treatment for more than four years and 11% had been on treatment for more than six years at the start of the study period (1 January 2007). Mean follow-up was 22 (± 13) months; for most patients (70%) follow-up ended on 31 December 2009 (end of study period). Mean time between INR measurements was 17 (± 5) days.

The estimates of INR control as determined by the cross-sectional and the longitudinal calculation methods were similar, within the therapeutic range INR (2.0-3.5) as well as in the target range INR (2.5-3.5) (*table 2*). The percentages of INR values within range were 74% and 59%, respectively, and the percentages of person-time within range were 77% and 61%.

In *table 3* and *table 4* various subgroup analyses are shown for both ranges. Similar or somewhat more control of INR was observed when excluding the first 2-6 months of treatment from the longitudinal calculation; for the cross-sectional method results did not differ between the 2-6 and >6 month treatment period.
From 2007 to 2009, the level of INR control was stable when determined by the cross-sectional calculation method (73-75% of values were in range). However, INR control improved when determined by the longitudinal calculation method: from 72% of person-time in range in 2007 to 80% of person-time in 2009. Between study years no differences were observed in the mean INR result, number of measurements per patient, time between measurements or the distribution of measurements by time since the start of treatment.

DISCUSSION

The aim of this study was to compare two calculation methods for assessing INR control in order to enable comparisons of the Dutch setting to international studies with new oral anticoagulants. These calculations were performed on the same dataset and with the therapeutic as well as the narrower target range for AF. Overall, the two calculation methods gave similar estimates. The longitudinal method gave slightly higher INR control estimates, likely due to the fact that INR values were weighted by the amount of person-time (and patients are sent home for a longer period when INR values are stable and in range), whereas in the cross-sectional analysis each INR value was equally eligible for selection while more measurements are performed when INR values are out of range. Another study comparing the methodologies found that the longitudinal method yielded lower estimates than the cross-sectional method, which is in contrast to our study.[12] The reason for the lower TTR results from the longitudinal method in that study was not clear.

Table 3. *Percentages of test results (cross-sectional) and person-time (longitudinal) in the therapeutic range (INR 2.0-3.5) by age, VKA preparation, and treatment phase*

	Cross-sectional method – INR tests		Longitudinal method – person-years	
	INR (2.0-3.5) / total*	**(% within range)**	**INR (2.0-3.5) / total**	**(% within range)**
<60 years	606 / 808	(75)	449 / 587	(77)
60-69 years	1670 / 2209	(76)	1375 / 1760	(78)
70-79 years	3410 / 4499	(76)	2879 / 3723	(77)
≥80 years	3277 / 4548	(72)	2784 / 3673	(76)
Acenocoumarol	7606 / 10,315	(74)	6302 / 8260	(76)
Phenprocoumon	1357 / 1749	(78)	1185 / 1482	(80)
2-6 months	800 / 1075	(74)	484 / 755	(64)
> 6 months	8163 / 10,989	(74)	7003 / 8,986	(78)

***One measurement per patient per six months.**

Table 4. *Percentages of test results (cross-sectional) and person-time (longitudinal) in target range (INR 2.5-3.5) by age, VKA preparation, and treatment phase*

	Cross-sectional method – INR tests		Longitudinal method – person-years	
	INR (2.5-3.5) / total*	**(% within range)**	**INR (2.5-3.5) / total**	**(% within range)**
<60 years	470 / 808	(58)	349 / 587	(60)
60-69 years	1310 / 2209	(59)	1083 / 1760	(62)
70-79 years	2779 / 4499	(62)	2301 / 3723	(62)
≥80 years	2607 / 4548	(57)	2182 / 3673	(59)
Acenocoumarol	6030 / 10,315	(58)	4933 / 8260	(60)
Phenprocoumon	1136 / 1749	(65)	982 / 1482	(66)
2-6 months	604 / 1075	(56)	347 / 755	(46)
>6 months	6562 / 10,989	(60)	5567 / 8986	(62)

***One measurement per patient per six months.**

In the two to six months after start of treatment, the two calculation methods resulted in different estimates of INR control likely due to the difference in selection of measurements. In the cross-sectional method, the last INR value of each patient in the database within a prespecified time period was selected for analysis, i.e. relatively late in the treatment period. Assuming improvement of INR control over the 2-6 month treatment period, the resulting estimate is higher than when calculated using all measurements between 2-6 months of treatment, as was done in the longitudinal method. After six months of treatment, anticoagulation is more stable and this difference was no longer present.

We also observed improving INR control over the study years 2007 to 2009 by the longitudinal calculation method but not by the cross-sectional method. The reason for this difference is unclear.

As described in the Thrombosis Service reports,[9-11] INR control was better under phenprocoumon than under acenocoumarol.

This study was based on data from the Thrombosis Service Region Eindhoven which monitors anticoagulation treatment of about 10,000 patients each year, and is therefore among the larger services in the Netherlands. The proportions of patients with arterial indications (86-87%) and atrial fibrillation (60-65% of arterial indications) are similar to the national median proportions over the study years (83-85% and 62-66%, respectively). The percentages of phenprocoumon users in this particular centre were 18-19% over the study years, which is above the national median of 9-12%. The percentage of INR results within range are, however, representative of the Netherlands: 77-78% of INR test results from long-term

patients were within the therapeutic range compared with the national median of 78-80%.[9-11]

Besides a different method to calculate INR control, the trials with new anticoagulants used a different therapeutic range. In these studies, patients with atrial fibrillation received warfarin under a therapeutic range of INR (2.0-3.0) while the Thrombosis Service has an INR target range (2.5-3.5) and a broader therapeutic range (2.0-3.5). The target range is set higher than the internationally advised target range to prevent inadequate anticoagulation (INR <2.0).[13] Quality estimates obtained for the Thrombosis Service in our study were 74% of INR values and 77% of person-time within therapeutic range (2.0-3.5) and 59% of INR values and 61% of person-time within target range (2.5-3.5). The trials reported 64%,[3] 62%[4] and 55%[5] of person-time within therapeutic range INR (2.0-3.0). Hence, compared with the target range in the Thrombosis Service, two of three trials reported slightly higher INR control.[3,4] However, given the broader therapeutic range in the Netherlands and the fact that these studies used selected populations, it may be more appropriate to conclude that the level of INR control was similar for the different settings. Although they used various calculation methods, INR ranges and indications for anticoagulation, other studies on Dutch data have reported estimates which are lower or equal to the estimates calculated in this study.[7,14-22]

In conclusion, the cross-sectional and longitudinal methods to assess INR control during anticoagulant therapy show similar results. Hence, the difference in calculation method is not a major limitation for comparing trial results with Dutch clinical practice.

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CASE REPORT

High-dose methylprednisolone-induced hepatitis in a patient with multiple sclerosis: A case report and brief review of literature

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ABSTRACT

Toxic hepatitis is a rare but serious complication of high-dose prednisolone treatment. We report a case of high-dose prednisolone-induced acute hepatitis in a 48-year-old woman suffering from multiple sclerosis that recurred after repeated administration. Timely recognition is paramount to avoid this complication. This report includes a brief review of the literature on methylprednisolone-induced hepatitis.

KEYWORDS

Hepatitis, methylprednisolone, multiple sclerosis

INTRODUCTION

Multiple sclerosis is a chronic recurrent inflammatory disease of the central nerve system. It follows a heterogenic clinical course due to multifocal demyelination and inflammation resulting in frequent relapses.[1] First-line treatment of relapsing multiple sclerosis is high-dose intravenous administration of methylprednisolone.[2] The beneficial action of methylprednisolone is pleiotropic and most side effects such as truncal weight gain and osteoporosis occur after prolonged administration of the drug. Acute side effects include hyperglycaemia, fluid retention and mental changes such as euphoria and insomnia.[3,4] Methylprednisolone-induced hepatotoxicity is rare but recurs on repeated administration and may be associated with a poor outcome.[5] We describe a patient suffering from multiple sclerosis in whom repeated administration of methylprednisolone led to recurrence of hepatotoxicity.

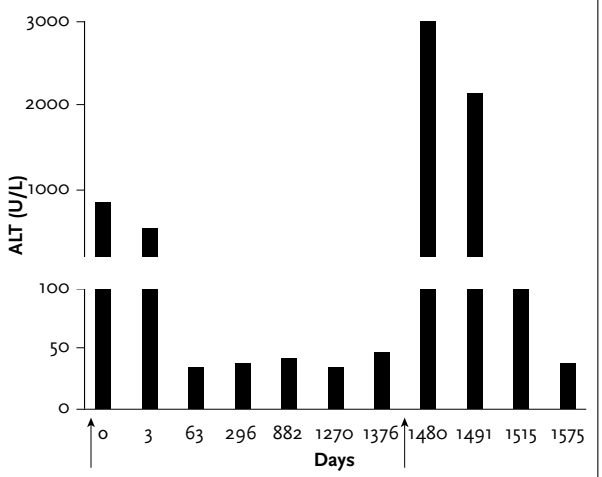
CASE REPORT

A 48-year-old woman was admitted to the emergency room in 2012 because of abdominal pain and nausea. In 1998 she was diagnosed with multiple sclerosis on the basis of clinical and radiological findings. In 2008, she was treated with a three-day course of methylprednisolone (1000 mg intravenously) because of a relapse of multiple sclerosis. This led to a pattern of grossly elevated liver enzymes (alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT)), two months following the treatment. The ALAT values returned to normal within four months. In 1998, 2001 and 2002 the patient was treated with a relatively low dose of dexamethasone orally (200 mg/day, 5 days) following a relapse of multiple sclerosis but this did not cause any elevation of the liver enzymes. On admission in May 2012, she reported similar symptoms to the earlier episode of hepatitis in 2008. Laboratory findings were now as follows: ALAT 3028 U/l (normal <35 U/l); ASAT 2384 U/l (<30 U/l); total bilirubin 29 μmol/l (normal <17 μmol/l); lactate dehydrogenase 1037 U/l (normal <490 U/l); gamma glutamyltransferase 182 U/l (normal <35 U/l); thrombocytes 127*10^9 E/l (normal 150-400*10^9 E/l); leucocytes 4.5*10^9 E/l (normal 4-10*10^9 E/l); haemoglobin 9.2 mmol/l (normal 7.5-10.0 mmol/l) and C-reactive protein 5.7 mg/l (normal <5 mg/l).

Some 19 days prior to admission she had been treated with a three-day course of methylprednisolone because of an exacerbation of multiple sclerosis. She was admitted and with conservative management the pattern of elevated liver enzymes eventually resolved. A summary of the course of the ALAT levels with time is shown in *figure 1*.

Alternative diagnoses for the onset of hepatitis were eventually ruled out. The patient denied any use of alcohol and was not taking any other medication. Serological

Figure 1. *Relation between alanine aminotransferase and methylprednisolone infusion*



The time course of alanine aminotransferase values in our patient. T values indicate time in days. The arrows indicate the timing of the methylprednisolone infusions. Note that ALAT increases in response to methylprednisolone infusions but returns to baseline values subsequently.

tests for hepatitis B, C, Cytomegalovirus, Epstein-Barr virus, Varicella Zoster and Herpes Simplex virus were all negative. Autoimmune hepatitis was excluded by a negative test of antinuclear antibodies, as well as negative tests for anti-smooth muscle antibodies. Tests for ferritin and transferrin saturation were normal. Abdominal ultrasound was compatible with mild liver steatosis.

Although histological confirmation is lacking, the combination of clinical and laboratory information suggested the diagnosis of methylprednisolone-induced toxic hepatitis. Indeed the unintended rechallenge four years after the first episode with ensuing hepatitis corroborates this hypothesis. We obtained a score of nine points (definitive adverse drug reaction) using the Naranjo scale.

DISCUSSION

We report a severe idiosyncratic toxic hepatitis from high-dose methylprednisolone. The side effect in this patient was specific for methylprednisolone but not for other steroids such as dexamethasone. An unintentional rechallenge test in 2012 confirmed the association between methylprednisolone and the onset of toxic hepatitis.

In order to obtain a comprehensive overview of high-dose methylprednisolone-induced hepatitis in multiple sclerosis we performed a literature search for PubMed articles for the years 1966-2012 using the keywords: multiple sclerosis, high-dose prednisolone intravenously, high-dose intravenous methylprednisolone, methylprednisolone, glucocorticosteroid, toxic hepatitis, liver toxicity, hepatotoxicity, acute hepatitis, hepatitis and the MESH terms: hepatitis and multiple sclerosis. Additional articles were obtained through citation snowballing to locate primary sources. A total of five case reports were identified (*table 1*). It is remarkable that all the case reports written about this topic involve women. This could be explained by the higher incidence of multiple sclerosis in women (3.6 cases per 100,000 person-years) compared with men (2.0 per 100,000 person-years). In addition, the female-to-male multiple sclerosis ratio has increased over the last years.[2]

In the reported cases, hepatotoxicity occurred three days to six weeks after intravenous methylprednisolone therapy. ALAT normalised in two weeks to four months after discontinuation of the drug. One patient was treated with glycyrrhizin in addition to withdrawal of methylprednisolone. Glycyrrhizin is an agent that is thought to protect against acute liver injury.

Drug-induced hepatotoxicity is usually idiosyncratic by nature. This reflects tissue injury that occurs without warning and is determined by individual susceptibility.[5] There are two types of idiosyncratic reactions: immunoallergic and non-allergic (metabolic). Immunoallergic reactions are dose-dependent and have a short latency period. Metabolic reactions seem to be dose-independent and have a variable latent period (days to months) to inception of injury.[6,7] Patients may develop hepatotoxcity even six months after discontinuation of a drug.[8] A non-allergic reaction occurs in 0.01% to 1% of patients exposed to the drug. Genetic as well as environmental factors play a role in the occurrence of an idiosyncratic reaction.[9] According to the literature, rechallenge after recovery of hepatotoxicity might not consistently reproduce the injury.[8] This supports the fact that environmental factors play an important role and explains why not all patients included in this review developed hepatitis after the first course of methylprednisolone therapy.[9] Interestingly, some suggest that the interval between pulsed methylprednisolone treatment and onset of elevated ALAT declines after the second episode of hepatitis. This has not been reported in other case reports and is difficult to understand given the non-allergic nature of the side effect (*table 1*).

Toxic hepatitis due to high-dose methylprednisolone therapy is a rare adverse event. It requires exclusion of alternative diagnoses and timely recognition of this drug-related reaction is important to allow stopping of the drug.[5] According to a survey of all liver transplantations in the USA between 1990 and 2002 (n=2.291) some 15% (n=375) were due to drug-induced acute hepatic necrosis.[10] There are no cases in the collated literature that document liver transplantation resulting from methylprednisolone-induced hepatitis.

Table 1. *Methylprednisolone-induced hepatitis in multiple sclerosis patients: review of five cases*

Reference	Age/ Sex	Type of steroid	Course	Dose and duration of treatment	Max. ALAT[a]/ ASAT[a] (U/l)	Max. GGT[a]/ ALP[a]	Time between treatment and elevation of liver tests	Histology	Concomitant treatment	Follow-up	Rechallenge
Furutama et al.[13]	11/F[a]	MP[a]	1st	1g iv/daily, 3days	800 / 278	36/NM	1-5 weeks	NM	No	Normalisation of liver tests 2 weeks after MP discontinuation, bed rest and treatment with glycyrrhizin	Yes, twice
		MP	2nd	NM	800/NM	NM/NM	1-5 weeks	NM	No	NM	
		MP	3rd	NM	820/NM	NM/NM	1-5 weeks	NM	No	NM	
Das et al.[14]	48/F	MP	3rd	NM	1600/1450	NM/200	6 weeks	Striking infiltration of parenchyma by lymphocytes, eosinophils and plasma cells	No	Normalisation of liver tests after MP discontinuation	Yes, once
		MP	4th	NM	NM	NM	3 weeks		NM	Normalisation of liver tests after MP discontinuation	
Fernández et al.[5]	57/F	MP	1st	1g iv daily, 3 days	2685/1328	71/115	3 days	Acute hepatitis with lytic necrosis and ceroid-laden macrophage hyperplasia	no	Normalisation of liver tests 3 months after MP discontinuation	Yes, twice
		MP	2nd	1g iv daily, 3 days	41/883	NM/NM	5 days		NM	Normalisation of liver tests 3 months after MP discontinuation	
		MP	3rd	1g iv daily, 3 days	1328/2685	56/115	NM		NM	NM	
Gutkowski et al.[6]	24/F	MP	2nd	500 mg iv daily, 6 days	1740/900	50/186	4 weeks	No biopsy (low PT)	Beta-feron	Normalisation of liver tests 3 months after MP discontinuation	Yes, once
			3rd	500 mg iv daily, 6 days	1129/1488	168/164	4 weeks		No	Normalisation of liver tests after MP discontinuation	
Hofstee et al.[15]	46/F	MP	2nd	1g iv daily, 3 days	1095/755	156/140	6 weeks	No biopsy	No	Normalisation of liver tests 4 months after MP discontinuation	Yes, twice
			3rd	1g iv daily, 3 days	1600/900	NM/NM	Few weeks	No biopsy	No	<4 months	
			4th	1g iv daily, 3 days	2350/950	NM/NM	Few weeks	No biopsy	No	<4 months	

ALP = alkaline phosphatase; ALAT = alanine aminotransferase; ASAT = aspartate aminotransferase; F = female; GGT = β-glutamyltransferase; M = male; MP = methylprednisolone; NM = not mentioned.

Although hepatitis is a rare side effect, awareness of this toxicity is important so that repeated exposure to methylprednisolone can be avoided. In acute exacerbations of multiple sclerosis, pulsed methylprednisolone treatment has shown to have a short-term benefit on the speed of functional recovery.[11] Patients who fail to recover after methylprednisolone treatment could be treated with therapeutic plasma exchange.[12] This might be an alternative therapy for patients developing acute hepatitis on methylprednisolone. Other options include IFN-beta which has been shown to reduce relapse rates.[11]

There is no screening model that predicts idiosyncratic hepatotoxicity. Therefore, monitoring of serum ALAT following high-dose methylprednisolone treatment

should be considered in order to prevent idiosyncratic hepatotoxicity.

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Referred shoulder pain in a patient with small cell lung cancer

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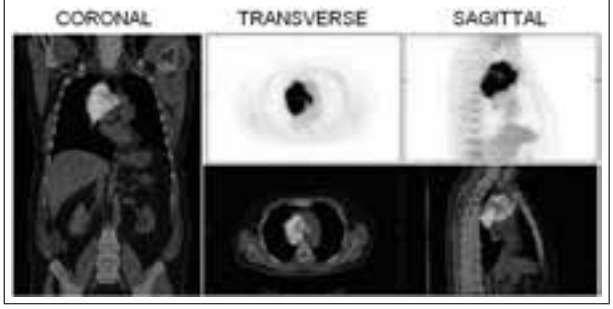
CASE REPORT

A 60-year-old man with no relevant medical history presented at the respiratory outpatient clinic with shortness of breath. Chest X-ray showed a large mass in the upper mediastinum and the patient was referred for FDG-PET/CT for staging of suspected lung cancer. FDG-PET/CT showed a large central tumour in the right lung attached to a large mediastinal mass (both FDG avid), without distant metastases (*figure 1*). During bronchoscopy a large ulcerating tumour was seen at the carina, partly obstructing the right main bronchus.

Histology revealed small cell lung cancer (SCLC) and the patient was staged as having limited disease, cT4N2M0 (International Association for the Study of Lung Cancer, 7th edition). During the next two months, the patient was treated with chemoradiotherapy plus prophylactic brain radiation. Follow-up CT chest two weeks after completion of therapy showed partial remission of the tumour.

After eight months, the patient complained of fluctuating pain in both shoulders, decreased appetite and weight loss. Diagnostic procedures were repeated (X-rays of shoulders and lumbar spine and extensive biochemistry) and were considered normal. Chest CT showed further decrease of the thoracic tumour.

Figure 1. *FDG-PET/CT showing a large central tumour in the right lung attached to a large mediastinal mass (both FDG avid), without distant metastases*



WHAT IS YOUR DIAGNOSIS?

See page 206 for the answer to this photo quiz.

PHOTO QUIZ

A red eye on the intensive care unit

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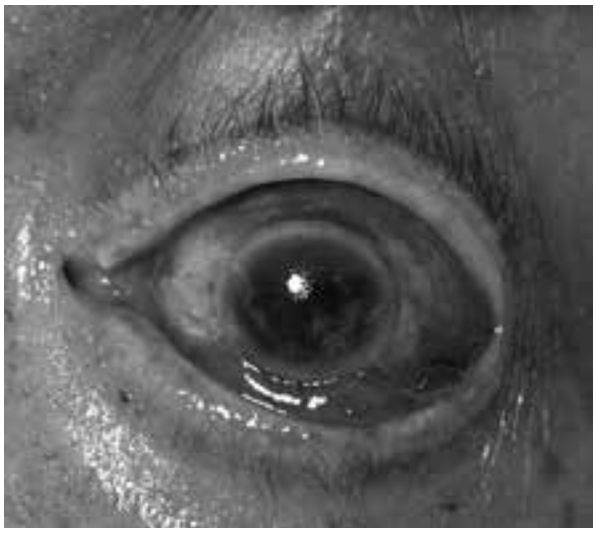
CASE REPORT

A 82-year-old man was admitted to the Intensive Care Unit (ICU) because of respiratory insufficiency. He was intubated, mechanically ventilated and resuscitated with large amounts of fluids. Because of chronic corticosteroid usage (prednisone 5 mg three times a day) for rheumatoid arthritis, a *Pneumocystis jirovecii* pneumonia (PJP) was suspected and treatment with high-dose trimethoprim-sulfamethoxazole (TMP-SMX) (1920 mg three times a day) and prednisone (40 mg twice a day) was initiated. A bronchoalveolar lavage (BAL) was performed and polymerase chain reaction (PCR) for PJP was positive. On the 12th day a red eye was observed (*figure 1*).

Figure 1. *The left eye on day 12: local conjunctival oedema, hyperaemia, subconjunctval bleeding, and local opacification of the cornea*



WHAT IS YOUR DIAGNOSIS?

See page 207 for the answer to this photo quiz.

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PHOTO QUIZ

A young woman with facial oedema

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CASE REPORT

A 23-year-old woman of Surinam-Hindu origin presented to our clinic with a two-week history of fatigue, headache, constipation and joint pain. Since four days she noticed a swollen face. She had no medical history, was not on any medication and the family history was unremarkable. Physical examination showed a moderately ill woman with a blood pressure of 130/60 mmHg, a pulse of 105 beats/min and a temperature of 37.8 °C. Her face was pale with periorbital oedema and oedema of the upper lip. Her thyroid gland was not enlarged.

Figure 1. *Before treatment*

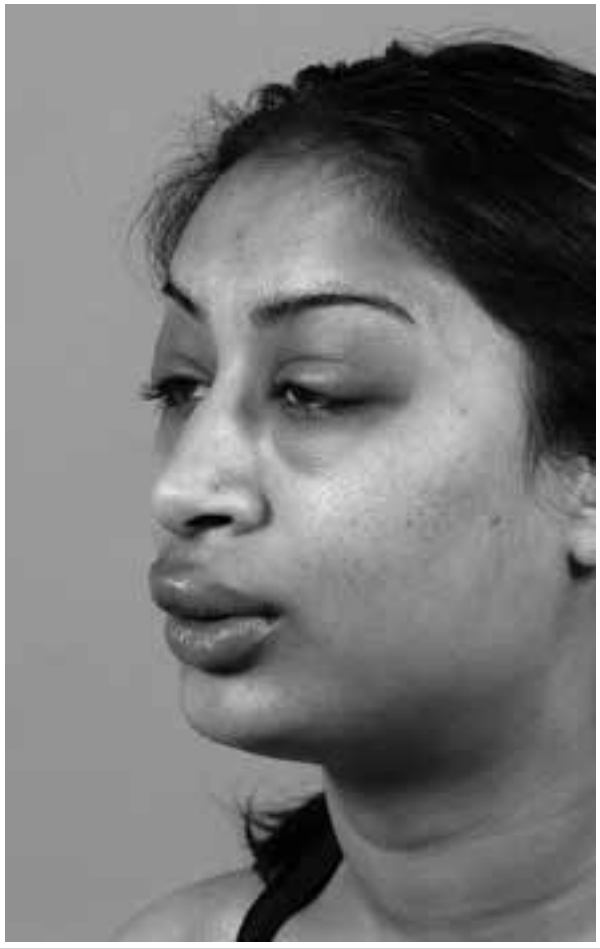

Figure 2. *After treatment*

WHAT IS YOUR DIAGNOSIS?

See page 208 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 203)

REFERRED SHOULDER PAIN IN A PATIENT WITH SMALL CELL LUNG CANCER

DIAGNOSIS

Repeated FDG-PET/CT displayed enhanced FDG uptake in extremely enlarged adrenal glands (11 x 11 cm on both sides) and focal FDG uptake in the lumbar spine (L5), consistent with metastases (*figure 2*). The adrenal glands could not be palpated at physical examination.

Endoscopic ultrasound guided fine-needle aspiration of the left adrenal gland was positive for metastasis of small cell lung cancer. The patient was thus diagnosed with extensive disease and treated with carboplatin/etoposide. Subsequently, the adrenal lesions decreased considerably in size on CT. Simultaneously, the pain in the shoulders disappeared. Both the time relation and the absence of local abnormalities of the shoulders support the hypothesis that the adrenal metastases caused the pain, as a result of excitation of the diaphragm. Thus, the pain in the shoulders is to be considered 'referred pain'.[1]

Shoulder pain can be attributed to a wide array of causes as shown in *table 1*.[1] Referred shoulder pain has been documented to be due to several aetiologies, one of which is diaphragmatic excitation related to several documented causes as shown in *table 2*.[1] Viscera abutting against the pleural or peritoneal surface of the diaphragm can cause referred pain to the ipsilateral shoulder. Excitation of the diaphragm or the adjacent areas of pleura or peritoneum will stimulate the roots originating from the phrenic nerves, i.e., the third, fourth, and fifth cervical nerves. Since these nerves innervate the skin of the neck, supraclavicular area, and shoulders, pain may be perceived in these areas.[2]

Figure 2. *PET/CT displayed enhanced FDG uptake in extremely enlarged adrenal glands (11x11cm on both sides) and focal FDG uptake in the lumbar spine (L5), consistent with metastases*

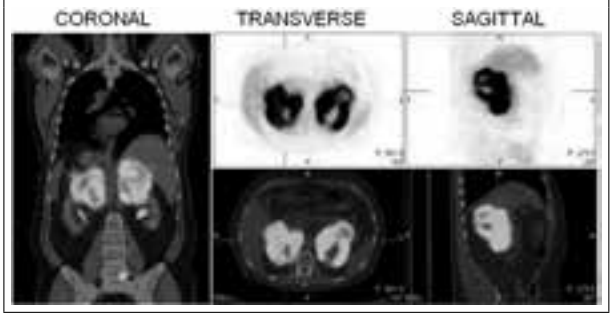

Table 1. *Causes of shoulder pain*

Trauma (contusion, fracture, rupture, AC-joint separation or injuries, rupture of rotator cuff)
Infection and inflammation (arthritis, capsulitis, bursitis, tendinitis)
Impingement syndrome
Anterior and posterior shoulder instability
Shoulder-hand syndrome
Local arterial, venous or lymphatic occlusion
Thoracic outlet syndrome
Hyperabduction syndrome
Costoclavicular syndrome
Myalgias and arthralgias
Psychogenic pain
Sleep dysesthesias
Congenital or developmental abnormalities
Neoplasm, primary or metastatic
Referred pain

Table 2. *Documented causes of referred shoulder pain*

Apical lung cancer (Pancoast's syndrome)
Cervical radiculopathy and brachial neuritis
Angina pectoris / myocardial infarction, or both
Diaphragmatic irritation:
- Biliary disease
- Myocardial infarction
- Blood or gas in peritoneal or pleural cavity
- Subphrenic abscess
- Splenic trauma
• Lower lobe pleuropulmonary inflammation
• Neoplasm
→ **Adrenal metastases**

To the best of our knowledge this is the first documentation of adrenal gland metastases as a cause of referred shoulder pain. This case exemplifies the potential role of FDG-PET in restaging of lung cancer,[3] as repeated physical examination and diagnostic procedures of the thorax and bones did not reveal a diagnosis. Eventually, the FDG-PET/CT clarified the pain in the shoulders most likely to be referred pain due to diaphragmatic excitation by the adrenal metastases.

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ANSWER TO PHOTO QUIZ (PAGE 204)

A RED EYE ON THE INTENSIVE CARE UNIT

DIAGNOSIS

The ophthalmologist was consulted and examination revealed incomplete eyelid closure (lagophthalmos), conjunctival oedema (chemosis) and subconjunctival bleeding. Diagnosis was exposure keratopathy with corneal abrasion secondary to lagophthalmos due to chemosis. The differential diagnosis was broad and included peripheral ulcerative keratitis in the context of a flare of his rheumatoid disease and microbial/viral keratitis among others. The first seemed less likely because the patient received high-dose corticosteroids and examination did not reveal any signs of vasculitis or specific infectious symptoms such as dendritic lesions in the corneal epithelium due to a *Herpes* infection.

The patient was treated with chloramphenicol 0,5% eye drops four times daily for seven days, oculentum simplex and application of a moisture chamber. During his ICU stay the condition of his eyes improved and when he recovered he turned out to have clear sight.

DISCUSSION

Ocular disorders most prevalent in ICU patients are chemosis (9% to 80%) and exposure keratopathy (3.6% to 60%). There are multiple reasons for critically ill patients to develop chemosis, e.g. fluid overload, increased capillary permeability, low plasma oncotic pressure and compromised venous return from the ocular region due to positive pressure ventilation. Furthermore, sedation compromises protective eye reflexes and masks ophthalmological symptoms such as pain. Lagophthalmos, frequently caused by chemosis, may lead to ocular surface desiccation and corneal abrasion.

Early diagnosis and treatment can be crucial, since exposure keratopathy may progress to microbial keratitis, corneal ulceration, perforation, scar formation and eventually permanent visual loss. When awake, vision loss and pain in the eyes cause discomfort and are a risk factor for delirium, associated with adverse outcome. Moreover, good sight highly contributes to the quality of life in the long term.

Exposure keratopathy and its complications can be prevented by using simple protocols. In the literature a variety of protective techniques are mentioned, all achieving adequate closure or covering of the eyes to maintain corneal moisture. Although no statistically significant difference is reported between the different preventive measures, application of polyethylene covers or moisture chambers together with lubricating ointments seem to be most effective. However, unfortunately, this is not common practice in most ICUs .

Previously, each mechanically ventilated patient in our ICU was treated with 1 drop hypromellose 0.3% every four hours together with 30-45 degrees elevation of the head of the bed. Because of this case we reviewed the literature and introduced a protocol which included frequent ocular examination by nurses, the use of ocular gels instead of hypromellose drops for sedated patients and mechanical closure of the eyes in case of lagophthalmos.

In conclusion, the patient's eyes are easily overlooked on the ICU, while simple measures can prevent serious complications.

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ANSWER TO PHOTO QUIZ (PAGE 205)

A YOUNG WOMAN WITH FACIAL OEDEMA

DIAGNOSIS/DISCUSSION

Laboratory results showed an elevated level of thyroid-stimulating hormone (TSH, 65 mIU/l), a reduced level of free thyroxine concentration (9.1 pmol/l) and thyroid peroxidise antibodies (TPO-Ab) were present. The presence of TPO-Ab supports that an autoimmune thyroid disease (Hashimoto's disease) is the cause for the diagnosed hypothyroidism. Hashimoto's disease is the most common cause of hypothyroidism in iodine-sufficient regions. It is caused by cell- and antibody-mediated destruction of thyroid tissue.[1]

Hypothyroidism is a common disease and has well-known signs and symptoms. It can affect all organ systems. In our case the most remarkable signs were the cutaneous manifestations: the pale skin, periorbital oedema and oedema of the upper lip. This is called myxoedema. The term myxoedema, formerly used as a synonym for hypothyroidism, refers to the symptoms of the skin and subcutaneous tissues in patients with a severely hypothyroid state.[2] Myxoedema is rarely seen today. A possible explanation could be the fact that patients now seek medical attention in a much earlier phase of their disease.

Hypothyroidism causes an increased deposition of glycosaminoglycans, especially hyaluronic acid, in the dermis. The increased deposition causes mucinous oedema which is responsible for the thickened features and a puffy appearance. The main cause of the depositions of glycosaminoglycans is local expression of the TSH receptor.[3,4] Myxoedema is a non-pitting oedema and is apparent around the eyes, on the dorsa of the hands and feet and in the supraclavicular fossae. It can also cause thickening of the tongue and the pharyngeal and laryngeal mucous membranes.[2] A histologically similar deposit may occur in patients with Graves' disease, usually over the pretibial area.[3] The skin pallor is very common and results from both peripheral vasoconstriction and increased deposition of water and mucopolysaccharides in the dermis, which alters the refraction of light.[5]

We treated our patient with oral administration of synthetic thyroxine, levothyroxine 50 mcg daily to reach an euthyroid state. This can easily be accomplished in almost all patients and appropriate treatment reverses the clinical manifestations of hypothyroidism.

After four weeks our patient still had some complaints of constipation, weight gain and fatigue but overall she felt rather well. The facial swelling had completely disappeared. Her laboratory results still showed an elevated TSH level and a reduced free thyroxine concentration. We increased the dose of levothyroxine. A few months later she reached the euthyroid state and had no more complaints. Hashimoto's disease is a permanent condition, and lifelong therapy is necessary.

In conclusion autoimmune hypothyroidism was diagnosed. Myxoedema as seen in this case is rarely seen today but it is a typically presentation of hypothyroidism. The term should be reserved for description of the physical signs.[2]

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SPECIAL ARTICLE

The standardised mortality ratio is unreliable for assessing quality of care in rectal cancer

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ABSTRACT

Background: The standardised mortality ratio (SMR) for rectal or anal cancer was above average in a large tertiary referral centre for locally advanced rectal cancer in the Netherlands. The aim of this study was to investigate whether the increased SMR was indeed related to poor quality of care or whether it could be explained by inadequate adjustment for case-mix factors.

Methods: Between 2006 and 2008, 381 patients were admitted for rectal or anal cancer. The SMR score of this diagnostic group was 230 (95% CI 140 to 355), corresponding with 20 in-hospital deaths. The hospital dataset was merged with data from the Eindhoven Cancer Registry to obtain more detailed information.

Results: Patients admitted for palliative care only accounted for 45% (9/20) of the in-hospital mortality. In contrast to the high SMR, postoperative mortality was low, i.e. 2.6%. The majority of the rectal or anal cancer patients were diagnosed in and referred from another hospital. Referred patients more often had an advanced tumour stage, more often underwent resection and were more frequently treated with chemotherapy and/or radiotherapy than non-referred patients ($p<0.01$). Postoperative mortality rates for referred and non-referred patients were 2.9% and 1.9%, respectively.

Conclusions: The increased SMR appeared to be caused by the admission of patients who received palliative care only. Consequently, the SMR is unreliable for the assessment of quality of care in patients with rectal or anal cancer.

KEYWORDS

Quality of care, rectal cancer, standardised mortality ratio

INTRODUCTION

Quality of care has become increasingly important in the last decades. Nowadays, in the Netherlands, quality of care of colorectal cancer is measured by a number of indicators. These include hospital participation in the Dutch Surgical Clinical Audit (DSCA) (which registers outcome of colorectal surgery), data on the number of examined lymph nodes after resection, and whether a patient is discussed during a preoperative multidisciplinary team meeting.

However, also other quality of care indicators are currently used, such as the hospital standardised mortality ratio (HSMR).[1] This measure is calculated by dividing the number of observed deaths in a given hospital by the number of patients that would be expected to die there. The national HSMR reference value is 100, hospitals with a higher score are supposed to have performed worse while the reverse would be true below 100. The HSMR is a hospital-wide measurement including several diagnostic groups (50 for the Dutch model) which are responsible for 80% of the in-hospital mortality. One of the 50 diagnostic groups comprises patients admitted for rectal or anal cancer. For each diagnostic group, a separate standardised mortality ratio (SMR) is calculated. The different SMR results could be interpreted and used separately or aggregated to create the overall hospital HSMR.

For each Dutch hospital, the HSMR is published by the Dutch Health Care Inspectorate (IGZ) in December 2011. However, the validity of HSMRs and their accuracy to reflect quality of care is heavily contested. Some have suggested that the HSMR is an appropriate measure to monitor hospital quality of care,[1-3] while others stated that it should not be used as a performance and/or quality indicator.[4-9] The consequences of making invalid measures

public could provoke an unjustified good or bad hospital reputation, groundless sanctions or rewards, disturbed collaborations between health care providers as well as damage to the public confidence. The HSMR calculation should be valid and accurate before making results public or incorporating in policy decision making.

In the last years, the SMR for rectal or anal cancer appeared to be increased in the Catharina Hospital. For a specialised centre for patients with locally advanced rectal cancer[10] this is very concerning. The aim of the present study was to investigate whether the increased SMR for rectal or anal cancer was indeed related to poor quality of care or whether it could be explained by inadequate adjustment for case-mix factors.

MATERIALS AND METHODS

Patients and data

Between 2006 and 2008, there were 484 admissions (381 patients) for rectal or anal cancer in the Catharina Hospital Eindhoven in the Netherlands. Of this diagnostic group about 20 patients died while a maximum of nine patients were expected to die based on the national average. The 11 excess deaths resulted in a significantly increased SMR of 230 (95% CI 140 to 355). The national SMR reference value is 100, scores above this value indicate that more patients died than expected while scores below 100 indicate that less patients died than expected. In the Dutch HSMR model (calculated over the period 2006-2008), expected mortality was calculated adjusted for age, sex, primary diagnosis, urgency of admission, Charlson comorbidity index, month of admission, year of discharge, social deprivation and source of referral. However, continuous refinements are made yearly to the included variables in the HSMR model.[2] The SMR calculations are based on data from the National Medical Registration (LMR) which are provided by hospitals and gathered by the Dutch Hospital Data (DHD). The diagnosis of rectal or anal cancer is classified according to the International Classification of Disease, Tenth Revision (ICD-10) and included the following codes: C154.0, C154.1, C154.2, C154.3, C154.8, C230.4, C230.5, C340.6 and V10.06. Consequently, these codes comprise one of the 50 Clinical Classification System (CCS) groups for which SMRs are calculated, i.e. rectal and anal cancer.

The Eindhoven Cancer Registry collects data on all patients with newly diagnosed cancer in a large part of the southern Netherlands. The area comprises approximately 2.3 million inhabitants, six pathology departments, ten community hospitals (including the Catharina Hospital) and two radiotherapy institutions. Besides patient characteristics, tumour data such as site, differentiation grade and depth of penetration are recorded as well. Tumour differentiation grade is classified as: well differentiated, moderately differentiated, poorly differentiated and unknown differentiation grade. Both clinical (cT) and pathological (pT) tumour penetration depth are recorded. For patients who did not meet the minimum requirements for classification (such as physical examination for cT or surgery/biopsy for pT) the tumour stage was classified as N/A. Due to neoadjuvant chemotherapy and radiotherapy, a less advanced tumour stage or even the absence of a tumour might occur during pathological examination resulting in a pT0 classification. In addition, data on hospital of diagnosis and treatment are recorded as well so referral patterns could be investigated. Patients admitted directly to the Catharina Hospital (non-referred) were distinguished from those diagnosed in another hospital and subsequently referred to the Catharina Hospital (referred).

To investigate patient and tumour characteristics of the 381 patients diagnosed at the Catharina Hospital with rectal or anal cancer, data of the Eindhoven Cancer Registry were merged with the hospital dataset on the basis of gender and date of birth. Patients with multiple matches were checked manually and those with corresponding zip codes were included. Patients with more than one tumour who had multiple matches were manually checked as well to select the correct tumour. From the hospital dataset 363 patients could be matched and were included in the new database. The other 18 patients were admitted to the Catharina Hospital for a recurrent rectal or anal tumour of which the primary tumour was diagnosed outside the Eindhoven Cancer Registry. Data of these patients were obtained from the Netherlands Cancer Registry which contains all newly diagnosed cancer patients in the Netherlands. Moreover, medical records of patients whose tumours were diagnosed more than one year before the hospital admission date were manually checked to rule out that they had presented with a recurrent instead of a primary tumour. As all registered data on patient and tumour characteristics of the Cancer Registries include information on time of primary tumour diagnosis and not regarding local recurrence, some analyses did not comprise patients admitted for a recurrent cancer (n=50). If this was the case, results were based on analyses of 331 (87%) patients.

Data on in-hospital mortality were obtained from the hospital database including 381 patients with rectal or anal cancer. In addition, the hospital medical records of the 20 patients who died during the hospital stay were reviewed for additional information including the reason of the admission (diagnostics, treatment, or palliative care).

Statistical analysis

Differences between referred and non-referred patients were compared using a Student's t-test for continuous variables and using chi^2-test for categorical variables

and reported as percentages. Statistical analyses were performed using SAS/STAT® statistical software (SAS system 9.3, SAS Institute, Cary, North Carolina, USA). A p-value <0.05 was considered to be significant.

RESULTS

Patient and tumour characteristics

Fifty patients (13%) had a locally recurrent tumour. As mentioned previously, no data were available for these patients with respect to their recurrent cancer and were therefore not included in *table 1*. The mean age of the remaining 331 patients was 65 (±11) years and 61% were men. The majority of the patients (97%) were diagnosed with rectal cancer, whereas only 3% were admitted for anal cancer. More than half of the patients had a tumour stage T3 or T4 (both cT and pT) and approximately half of the patients had a moderately differentiated tumour.

Table 1. *Patient and tumour characteristics*

	Total (n=331)
Patient characteristics (%)	
Age	65±11
Male gender	61
Tumour characteristics (%)	
Tumour site	
- Rectal cancer	97
- Anal cancer	3
Depth of penetration	
- Pathological	
--- To/in situ	8
--- T1	6
--- T2	25
--- T3	42
--- T4	7
--- Unknown	2
--- NA*	10
- Clinical	
--- To/in situ	0
--- T1	4
--- T2	5
--- T3	27
--- T4	31
--- Unknown	31
--- NA*	2
Tumour differentiation grade	
- Well differentiated	7
- Moderately differentiated	51
Poorly differentiated	7
Unknown	35

Patients with recurrent disease not included. *Patients did not meet minimum requirements for classification.

Referred and non-referred patients

Of all 381 patients, 235 (62%) were diagnosed with cancer in another hospital and subsequently referred to the Catharina Hospital. Forty-six different medical centres referred patients to this hospital. Excluding patients with a recurrent tumour, 192 of 331 (58%) were referred. Of these 331 patients diagnosed with a primary rectal or anal tumour, referred patients were younger (64 *vs* 67 years, respectively) and had a more advanced tumour stage (pT3 + pT4; 57 *vs* 39%, respectively) compared with non-referred patients (p<0.01).

In-hospital mortality

During the study period, 20 (5.2%) patients died in the Catharina Hospital. *Figure 1* demonstrates in detail the in-hospital mortality (including patients with a recurrent tumour). Of these 20 patients, nine were referred from another hospital and 11 were primarily diagnosed in the Catharina Hospital. Two patients had a recurrent tumour, one referred and one non-referred patient. Nine patients were admitted to receive palliative care only: eight non-referred patients and one referred patient. So only 11 of the 20 deceased patients were admitted and treated with curative intent.

Table 2 presents the percentages of in-hospital mortality for the referred and non-referred patients who underwent resection and those who did not (recurrences not included). As no treatment data were available for recurrent tumours (n=50), the results are based on analyses without these recurrences (n=331). In total 192 (58%) of the 331 patients were referred, of whom 3.7% died within the hospital whereas 7.2% of the patients who were diagnosed in the Catharina Hospital died (p=0.15). Total postoperative mortality after surgical resection was low at 2.6% and did not differ between referred and non-referred patients, 2.9% and 1.9%, respectively (p=0.61). In contrast, in-hospital mortality of the non-resected patients was 17.9%. The

Figure 1. *Flowchart of patients who died within the hospital stay*

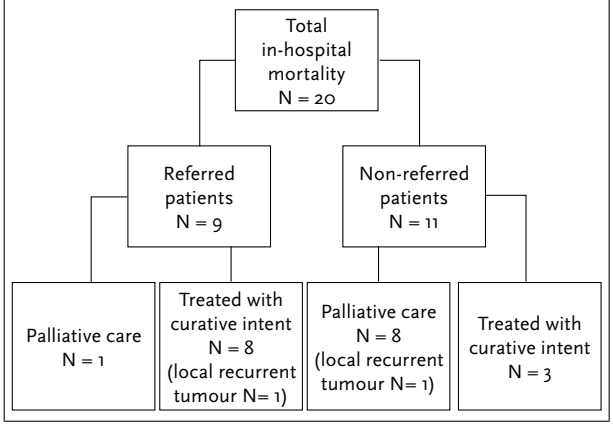


Table 2. *Percentage in-hospital mortality*

	Total (n=331)	Referred (n=192)	Non-referred (n=139)
	n (%)	n (%)	n (%)
Total (n=331)	17 (5.1)	7 (3.7)	10 (7.2)
Resected (n=275)	7 (2.6)	5 (2.9)	2 (1.9)
Non-resected (n=56)	10 (17.9)	2 (9.5)	8 (22.9)

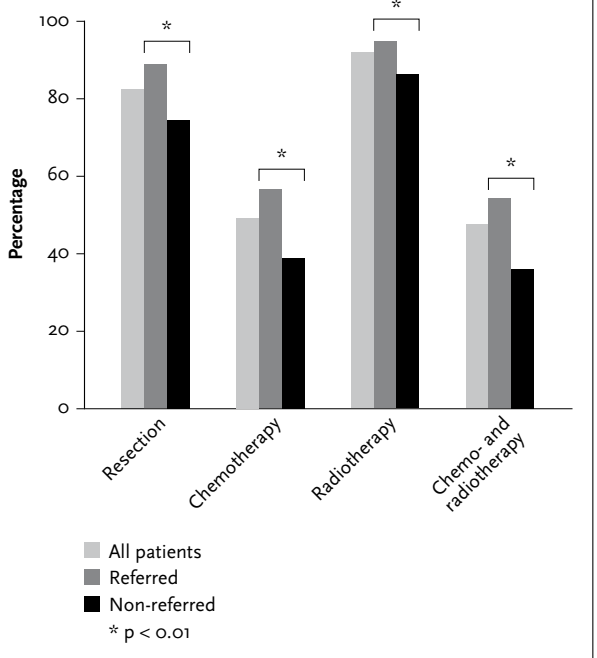
majority (60%) of this group were terminally ill and admitted for palliative care only.

In total, nine of the 20 patients (45%) died within the postoperative period due to surgical complications. Two patients (10%) were admitted due to acute need for gastrointestinal surgery and died as a result of a cardiovascular cause. The remaining nine patients (45%) were all admitted with end-stage disease and severe symptoms to receive palliative care only (described above).

Therapy

Of the 331 patients of whom treatment data were available, 275 patients (83%) underwent resection for the tumour. Of these surgically treated patients, 92% received radiotherapy, 50% chemotherapy and 48% of the patients both radiotherapy and chemotherapy (*figure 2*). Referred patients more often underwent surgical resection than non-referred patients (89 *vs* 75%, respectively; p<0.001) and more often received chemotherapy, radiotherapy or chemoradiotherapy (p<0.01 for all). Of the non-resected patients (n=56) two-thirds (68%) received chemotherapy or radiotherapy or both.

Figure 2. *Treatment of patients with rectal or anal cancer according to referral status. Percentage chemotherapy, radiotherapy, and chemo- and radiotherapy of patients who underwent surgical resection (n=275)*



DISCUSSION

Centralisation of complex, low-volume surgery in specialised centres to improve quality of care is an important issue in the current health care system. As a result, the Catharina Hospital has successfully provided specialised care for patients with locally advanced and locally recurrent rectal cancer for several years.[11-14] However, this seems to be in contrast with the SMR for this diagnosis group which appeared to be significantly increased for the period 2006-2008 in this hospital. The results of the present study demonstrated that patients admitted for palliative care accounted for approximately 50% of the in-hospital mortality for patients with rectal or anal cancer resulting in an increased SMR. Moreover, insufficient case-mix adjustment for the reason of admission and referral patterns is likely to negatively affect the SMR for this diagnostic group.

For a significant part, the increased SMR for rectal and anal cancer is explained by the admission of terminally ill patients who require or opt for end-of-life care in the hospital, since almost half (45%) of the deceased patients were admitted to receive such palliative care only. While these patients were all expected to die during the hospital stay, the predicted chance to die, based on the SMR adjustment model, is not calculated as (nearly) 100% for these patients. This leads to an underestimation of the in-hospital mortality and consequently to an increased SMR. Moreover, comparison of in-hospital mortality rates of resected (2.6%) and non-resected (17.9%) patients confirms the suggestion that the admission of patients who could not be cured increases the in-hospital death rate. Consequently, the reason of admission should be incorporated in the HSMR adjustment model or, even better, patients admitted for palliative care should be excluded when HSMRs or SMRs are calculated.

In addition to the hospitalisation of terminally ill patients, those with recurrent rectal or anal cancer might considerably affect the SMR as well. The Catharina Hospital is a specialised centre for the treatment of patients with local recurrence;[10] however, the extended anatomical resections cannot be compared with primary cases. Certain subsites of the recurrent tumour are a major problem in rectal cancer surgery such as a postero-lateral recurrence which is associated with 20% mortality within three months after surgery.[15] Irradical

resection rates and consequent cancer-related deaths are considerable among patients with recurrent rectal cancer. Furthermore, treatment of these locally recurrent cases is limited to very few centres in the Netherlands, of which the Catharina Hospital is by far the largest. This condition cannot be assessed within the framework of the SMR for rectal or anal cancer. We identified 13% of the rectal cancer patients included in our study as admitted for a recurrent tumour and patients with a recurrent tumour accounted for 10% of the in-hospital mortality.

The majority of the rectal or anal cancer patients were referred from other hospitals to the Catharina Hospital which serves as a tertiary centre for patients with a locally advanced and locally recurrent rectal carcinoma. The latter was confirmed by our results which demonstrated that the referred patients more often had an advanced tumour stage (T3 and T4) and a recurrent tumour compared with the patients who were primarily diagnosed in the Catharina Hospital. Referred patients living outside the local community usually have a different risk profile than patients who are admitted directly; they are more ill, have a longer length of hospital stay and have greater mortality rates.[16-19]

The Dutch adjustment model which was used to calculate the SMR for rectal cancer for the period 2006-2008 only included the Charlson comorbidity index to adjust for disease severity. A specific variable for disease severity was lacking. Recently, the model has been expanded by the addition of a detailed variable for disease severity. However, despite these changes to the model, the adjustment for severity of disease is still insufficient.[20] Moreover, transfer of severely ill patients is likely to reduce the HSMR or SMR for the referring hospital[21] while accepting these patients may lead to increased mortality rates.[16,22] Consequently, a tertiary referral centre will be 'punished' twice when a referred patient dies during the hospital stay; the referring hospital will be given a positive score for the high-risk patient who is leaving the hospital alive while the accepting hospital will be given a negative score when this patient dies. So large variation between hospitals in the admission of patients with advanced disease could result in diverse and conflicting in-hospital mortality rates. Consequently, publication of incorrect SMRs will cause more harm than good for hospitals as well as for patients as they could be denied admission to medical centres supplying the specialised care they require.

In contrast to the significantly increased SMR for rectal and anal cancer, the in-hospital mortality rate among patients who underwent surgical resection in the Catharina Hospital was low (2.6%) and in accordance with the national[23] and international[24,25] literature. Our results are also confirmed by recent mortality data which were established by the Dutch Surgical Colorectal Audit, DSCA. For the period 2009-2011, the hospital volume for rectal cancer surgery was more than 250 for the Catharina Hospital and the in-hospital mortality of 2% was below the average.[26] These data clearly demonstrate and confirm that our hospital serves as a centre of excellence for rectal cancer patients and provides good quality of care. The results of our study demonstrated that even for referred patients who often suffer from advanced disease, low mortality rates were accomplished. However, given the different risk profile between referred and non-referred patients and the fact that these patients affect in-hospital mortality of both the referring and accepting hospital, it is crucial that referral patterns are taken into account when SMRs are calculated. For example, intention-to-treat analysis may be considered in which mortality will count for the first hospital the patient was admitted to. Given the referral patterns due to concentration of specialised care, quality of care should be investigated at a regional level instead of a hospital level.

Quality of care is currently an important topic in health care; however, there remains considerable debate about which measures should be used to reflect quality of care.[27] The most common framework is that of Donabedian who conceptualised three quality of care dimensions, i.e. structure, process and outcome.[28] Direct outcome measures such as morbidity and mortality are most appealing to use for the evaluation of quality of care as they are relatively easy to use and usually widely available. However, as the results of our study confirm as well, the focus should not be solely on outcome measures such as in-hospital mortality. Structural elements such as hospital type, the availability of specific technologies/treatment options and the presence of health facilities such as hospices in the hospital area may have a significant impact on in-hospital outcome as this results in the admission or discharge of specific patients. In addition, outcome measures other than mortality should be considered as well. For rectal or anal cancer patients undergoing surgery, complication rates, reoperation rates, cancer recurrences, length of hospital stay and guideline-recommended treatment are related with quality of care and may also be considered.[23]

The additional information obtained from the cancer registry made it possible to explore potential patient and tumour characteristics of rectal and anal cancer patients which could affect in-hospital mortality. However, less detailed data were available for patients admitted for a recurrent cancer. Moreover, the exact method of SMR calculation was unfortunately confidential, so we were not able to re-calculate the SMR for rectal and anal cancer. Additional analyses should be performed with and without the aforementioned variables (i.e. tumour recurrence, palliative care, and referral patterns) to investigate the actual effects of these factors on SMRs.

In conclusion, the SMR for rectal and anal cancer was significantly increased which is in contrast with the low postoperative mortality rates for the patients who underwent surgical resection. Patients admitted for palliative care accounted for almost half of the in-hospital mortality. Given the inadequacies, the SMR for rectal and anal cancer is unreliable and should not be used to assess quality of care.

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SPECIAL ARTICLE

Teaching adjuvant endocrine breast cancer treatment to medical students

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ABSTRACT

Background: In undergraduate medical education, students are supposed to acquire knowledge and understanding about the basic principles of adjuvant breast cancer treatment. The best education method in this context is unknown. In this randomised study we assessed the effect of designing a patient education poster on knowledge, perceived participation and students' satisfaction compared with case-oriented education concerning endocrine therapy for breast cancer patients.

Methods: This study was conducted in the Bachelor Oncology Course for undergraduate students in Medical Science of the Radboud University Nijmegen Medical Centre. In the experimental group, students designed and created a patient education poster in small groups. In the control group, students answered case-based questions in small groups. Knowledge was tested at different moments using multiple-choice questions. To assess perceived participation and satisfaction, students filled out questionnaires.

Results: 329 students participated in the study. No difference in knowledge was observed between the experimental and control group. However, students in the control group reported a higher perceived participation and satisfaction compared with the students in the experimental group ($p<0.05$).

Conclusion: In this study, working on case-based questions was preferred compared with designing a patient education poster in terms of students' perceived participation and satisfaction. Working on case-based questions may be appreciated by medical students as most relevant for their future profession. We advocate more attention to the importance of patient education in the medical curriculum, to help students realise the relevance of this aspect of medical profession.

KEYWORDS

Breast neoplasms, competency-based education, medical oncology, undergraduate medical education

INTRODUCTION

Breast cancer is the most common life-threatening malignancy among women and the second most common cause of cancer death in women in the Western world.[1] Over the years, mortality has declined partly due to improved treatment modalities. One of these modalities is adjuvant endocrine therapy in hormone-sensitive breast cancer patients. In postmenopausal patients, adjuvant endocrine treatment with an upfront aromatase inhibitor (AI) or a switch therapy of tamoxifen and an AI is standard of care, while in premenopausal women adjuvant treatment with tamoxifen with or without ovarian suppression is recommended.[1] Due to the (rising) incidence of breast cancer a large proportion of medical professionals will be faced with breast cancer patients. Therefore, in the Medical Science Bachelor curriculum of the Radboud University Nijmegen Medical Centre (RUNMC), students are supposed to acquire knowledge and understanding about the basic principles of adjuvant breast cancer treatment, such as working mechanisms, (contra)indications and side effects. However, the question remains which instructional method is most effective?

Previous studies have indicated that cooperative learning is a very powerful method to process information.[2] Cooperative learning refers to all educational methods where students work together in groups in positive interdependence to accomplish shared learning goals. Working in small groups of 6-8 students improves the quality of discussions and the development of skills such as deep thinking and sharing of experiences.[3] Moreover, working in small groups, students feel that they participate more actively.[4] Active participation of students has shown to contribute to knowledge. Learning processes are stimulated by the fact that learners communicate and interact actively.[5]
To further enhance learning in small groups, it seems relevant that students can work on specific products such as posters, poster presentations, or concept maps.[6] Working on concrete products stimulates interaction between students, increases their knowledge about the subject and their self-confidence.[7]

However, to the best of our knowledge no studies are available that investigate the influence of working in small groups on a concrete product in medical oncology education. In this study we will assess the effect of working on a concrete product in small groups, viz. the design of a patient education poster, with respect to knowledge, perceived participation and students' satisfaction in a Bachelor course versus conventional sessions. Designing a patient education poster was chosen as it is a concrete and sensible task that was expected to stimulate deep learning as it required students to actively incorporate knowledge in order to summarise and rephrase this knowledge into plain, understandable language. The design of a patient education poster as a teaching method was regarded specifically appropriate in the context of adjuvant endocrine treatment, as proper patient education may improve adherence to endocrine treatment and, therefore, treatment outcome.

MATERIALS AND METHODS

Setting

In the conventional Bachelor curriculum of the RUNMC education is structured in blocks and organised in themes. Students visit lectures, work together in small groups and do assignments in order to deepen the different topics. Afterwards, they have the opportunity to visit a dedicated response session, where they can ask questions about the topics of the course. And lastly, they make a final assessment to finish the course. The part of the oncology course that was the subject of the present study focussed on adjuvant hormonal treatment of breast cancer patients, teaching working mechanisms, side effects, indications and outcome of tamoxifen and aromatase inhibitors.

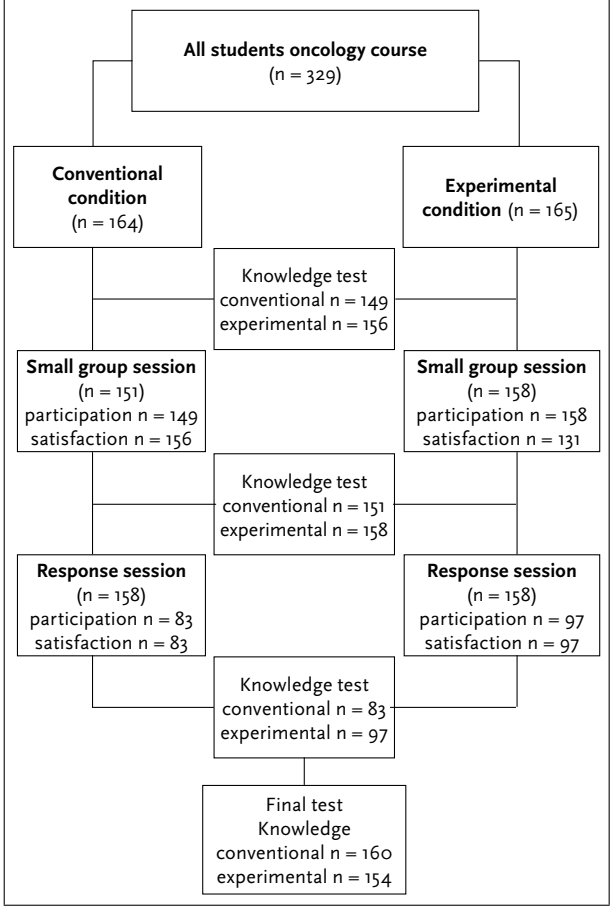
Respondents

A total of 329 undergraduate medical students (33.7% male, 66.3% female) of the RUNMC signed up for the mandatory second-year Bachelor Oncology Course in November and December 2010. During the first plenary lecture of the course, students were informed about the study. Students were randomised to attend either two conventional sessions on adjuvant endocrine treatment or two experimental sessions. The male-female ratio was equal in both groups. In both groups, the first session consisted of a mandatory small group session and the second session of a (non-mandatory) response session. Three hundred and nine students participated in the small group sessions and 180 of these students attended the response sessions (*figure 1*). The study was approved by the general coordinator of the course as well as the education management team of the RUNMC.

Procedure

In both the conventional and the experimental approach the first small group session lasted two hours, while the subsequent response session lasted one hour. During the first session in the conventional arm students were divided into small groups of five persons and, as a

Figure 1. *Study design*



group, had to answer questions based on patient cases concerning adjuvant endocrine breast cancer treatment with tamoxifen and the aromatase inhibitor letrozole. During the response session all the students assigned to the conventional arm had the opportunity to ask questions about the subject. In the experimental arm, in the first session the students designed a patient education poster on adjuvant endocrine treatment of breast cancer with either tamoxifen or letrozole in small groups of five students and prepared themselves to present the poster in the response session. During this response session students could read all the posters created by their colleagues. Two of the created posters per session – one on letrozole and one on tamoxifen – were selected to be presented. Afterwards, the posters were discussed and the students had the opportunity to ask questions.

To prepare for the sessions on endocrine treatment of breast cancer patients, all students were expected to read specific chapters in their textbook on adjuvant endocrine treatment and answer the accompanying questions.

The students participating in the study filled out questionnaires to assess knowledge, perceived participation and satisfaction (*figure 1*). Before the start of the course, the four medical oncologists who were teaching in the adjuvant endocrine treatment sessions were informed about the study and attended an oral presentation on the new teaching approach. They also received a written manual on the small learning groups and the corresponding response session. All teachers had several years of experience in both medical oncology and medical teaching. The teachers were allocated to teach in two conventional sessions and two experimental sessions.

Instruments

To assess knowledge, four tests with comparable items for knowledge testing were handed out: before the first session to assess prior knowledge, directly after the first and second session and in the final test of the whole oncology course (1.5 weeks later). To assess the effect of the new teaching approach on perceived participation and satisfaction, students filled out questionnaires just after the first and second session.

The tests to determine the students' knowledge were designed by a teacher in medical oncology. The course coordinator and an assessment expert were consulted. Each test consisted of six multiple-choice questions, which covered the predefined subjects of the small group learning (working mechanisms, (contra)indications and side effects of adjuvant endocrine treatment). Each of these questions gave a score of 1 point, the questions could be answered as correct or incorrect.

Based on a questionnaire by Kooloos *et al.*[8] a 19-item questionnaire was constructed to assess perceived participation and student satisfaction. This questionnaire was administered directly after the small group session to assess the perceived participation and satisfaction of the students about the small group session that they had just attended (Cronbach's alpha .89) and the response session, to assess the perceived participation and satisfaction of the students about the response group session that they just attended (Cronbach's alpha .94).

Statistics

For the statistical analyses, the statistical package for social sciences (SPSS) Windows version 18.0 was used. To determine knowledge, t-tests for repeated measures were performed. To assess differences between the experimental and control group variance analyses, one-way ANOVAs were conducted using the difference scores of the knowledge tests; $p<0.05$ was considered statistically significant.

To assess differences between the average scores for student satisfaction and perceived participation in the two teaching methods, one-way ANOVA analyses of variance were conducted. If differences between the teaching methods were observed, an independent t-test was performed to further assess those differences.

Post-hoc analyses were conducted to compare knowledge, perceived participation and student's satisfaction, attendance at the non-compulsory response session, and students' self-reported preparation for the sessions. Also, we compared the group of students who studied tamoxifen with the group of students who worked on letrozole.

RESULTS

Knowledge

In both the experimental and the control group knowledge significantly increased from the pretest to the final test. The highest scores were attained at the second post-test, directly after the response session. No difference was observed in knowledge between the experimental and the control group (*table 1*).

Post-hoc analyses showed that students who had attended the response session had higher scores on the final test than students who did not attend this session ($p<0.001$). No differences were observed on the pretest between the students who prepared for the sessions compared with those who did not read the textbook and answer the accompanying questions ($p=0.182$). However, the students who reported to have prepared themselves scored higher on the post-test directly after the response session ($p=0.039$) as well as on the final test ($p=0.001$).

The students who worked on the posters on tamoxifen showed higher scores on the post-test after the small group session and response session compared with the students who prepared for letrozole. On the final test, no differences were observed between the groups.

Table 1. *Average proportion of correctly answered questions on the four tests*

	Experimental group			Control group		
	N	Av.	SD	N	Av.	SD
Pretest first session	156	.67	.20	149	.69	.20
Post-test first session	158	.79	.15	151	.77	.18
Post-test second session	97	.89	.13	83	.89	.14
Final test	154	.72	.16	160	.71	.18

Perceived participation

The students in the conventional arm (working on the questions based on a case) reported a higher perceived participation during the small group session compared with the students in the experimental arm (working on the patient education poster) (p=0.037) (*table 2*). No differences in perceived participation were observed between the students who had prepared themselves compared with the students who had not prepared themselves for the sessions. Perceived participation was not significantly different between the tamoxifen and letrozole subgroups.

Students' satisfaction

The students in the conventional groups working on the cases showed higher satisfaction about the two educational sessions compared with the students in the experimental group, who worked on a poster (*table 3*). No differences in satisfaction were observed between the students who had prepared themselves compared with the students who had not prepared themselves for the sessions. Students' satisfaction was not significantly different between the tamoxifen and letrozole subgroups.

Table 2. *Average score for perceived participation in the first and second session*

	N	Av.	SD	df	F	p
First session, experimental group	158	14.84	2.33	307	4.41	.037*
First session, control group	151	15.34*	1.83			
Second session, experimental group	97	8.95	1.93	178	1.46	.228
Second session, control group	83	9.25	1.34			

*p<.05.

Table 3. *Average score for satisfaction in the first and second session*

	N	Av.	SD	df	F	p
First session, experimental group	131	36.90	8.27	260	62.40	.000*
First session, control group	131	46.66*	7.60			
Second session, experimental group	97	41.02	11.40	178	15.18	.000*
Second session, control group	83	47.33*	10.11			

*p<.05.

DISCUSSION

In this study, we observed that the acquired knowledge on adjuvant endocrine breast cancer treatment proved to be similar for students working on an education poster (experimental group) and students answering questions based on a case (control group), while student satisfaction was lower in the experimental group working on posters. Perceived participation was lower in the experimental group for the small group session, although no differences in perceived participation in the response session were observed between the conventional and the experimental group. Although our study focused on educational sessions in an oncology course, the results may be of direct relevance for other medical courses as our prestudy hypothesis – working together in small groups on a specific product increases learning satisfaction, perceived participation, and knowledge – would in principle be applicable to a broad range of medical topics.[3-5] However, despite our expectations, this hypothesis was not confirmed in our study.

Two major reasons can be identified that could explain the discrepancy between the results of the study and our pre-study hypothesis. First of all, for these medical students, creating a patient education poster may have been new for them and they may have felt uncomfortable about it. More specifically, working on a case may have been regarded by the students as more relevant for their future profession than creating a patient education poster. Applying their medical knowledge to solve clinical problems may have seemed more 'medical doctor-like' than patient education.

Interestingly, however, according to the CanMeds roles, patient education is one of the main competences of a health care professional.[8] CanMeds is an educational framework identifying and describing seven roles of health care professionals that would lead to optimal health care delivery and outcomes. This framework of core competencies includes the different roles that physicians fulfil in their daily practice, namely the roles of Medical Expert, Communicator, Collaborator, Health Advocate, Manager, Scholar and Professional. In the context of endocrine breast cancer treatment, patient education is indeed a very relevant competence, as the benefits of endocrine treatment in terms of disease recurrence and survival require long-term adherence to the medication regimen. Previous studies indicate that 5-32% of the patients discontinue endocrine treatment or skip or lower their doses in the course of time.[9,11-14] Proper patient education may improve adherence and, therefore, treatment outcome.

Currently, the CanMed framework forms the basis for objectives of medical training and provides the standard for continuing professional development. However, students in the current course may not have been familiar with these CanMed roles. In fact, patient education was not explicitly mentioned as a formal learning objective of the course.

A second reason that could explain the discrepancy between the results of the study and our pre-study hypothesis concerns the role of assessment driven learning. Assessment driven learning implies that students prepare mainly for the assessment they have to do.[10] Student's perceptions about the way they will be assessed determine their choices for and emphasis on specific learning strategies en learning activities.
In our study, students were preparing mainly for multiple-choice questions directly covering the (knowledge-based) subjects of the course. Although having to think about the presentation of the poster and rewording medical terminology in plain language could have stimulated deep learning,[3] in practice, students may have felt that creating a poster distracted them from their primary learning task. The purpose of the education poster, related to the knowledge-based assessment, may not have been clear to them. Therefore, our results could not only be explained by the perceived relevance of the learning task for the students' profession in the far future, but also by the relevance for the assessment of the course in the near future.
Of note, students who attended the non-obligatory response session had higher scores on the final test than students who did not attend these sessions. Also, students who prepared themselves by making the self-assignment had higher scores on the post-test directly after the second session. These results may not only be explained by more exposure to the content of the course, but also by a higher intrinsic motivation of the students. The students attending the response session and the students who made the self-assignment may have been more motivated than the students who did not attend the response sessions or made the self-assignment. Unfortunately, motivation was not tested in this study and may be relevant to incorporate in future studies on the effect of new educational approaches.

In conclusion, according to the results of our study, to teach medical students the principles of adjuvant endocrine treatment of breast cancer patients, working on case-based questions must be preferred compared with creating a patient education poster in terms of student's participation and satisfaction. This may be applicable to other medical topics, too. By working on case-based questions, students recognise the importance for their future profession more easily and they feel comfortable with this way of learning. However, as patient education will be an important part of their future profession, we advocate that this perspective should be introduced more explicitly as a learning objective in the educational as well as in the assessment programme.

Disclosures

Data from this manuscript were presented at the Netherlands Association of Medical Education Congress in Egmond aan Zee, the Netherlands, on 18 November 2011.

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LETTER TO THE EDITOR

Dapsone for the treatment of chronic IgA vasculitis (Henoch-Schonlein)

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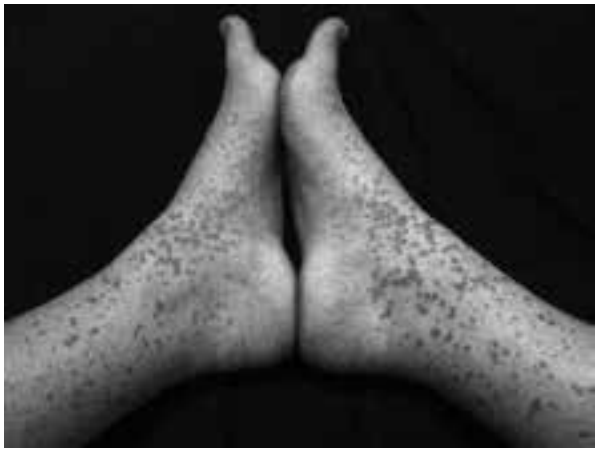
Dear Editor,

IgA vasculitis (IgAV; Henoch-Schonlein) in adults is a rare disease belonging to the category of immune complex small-vessel vasculitides.[1,2] Patients present with purpura, nephritis, arthritis and/or abdominal pain. A chronic form of IgAV occurs predominantly in patients with isolated cutaneous involvement.[3] The aetiology of this chronic form of IgAV is unknown. In 40% of patients a trigger is identified such as infections, drugs, toxins and/or malignancies.[3-5] Furthermore, IgAV can be associated with other diseases such as liver disease, inflammatory bowel disease, and/or ankylosing spondylitis. Treatment of IgAV with internal organ involvement consists mainly of corticosteroids. Unfortunately, this is ineffective for the chronic cutaneous form of IgAV.[3,6]

A 21-year-old woman presented to the outpatient department of internal medicine because of chronic skin lesions of the legs. The lesions had already been present for two years. This was a symmetrical, non-pruritic rash which dominated on the extremities and which was influenced by orthostasis. Her medical history showed recurrent urinary tract infections. Apart from oral contraceptives she was not on any medications. On physical examination no abnormalities were found besides a non-palpable skin rash of the legs (*figure 1*). A skin biopsy was taken which showed a leukocytoclastic vasculitis with depositions of IgA and C3. A diagnosis of chronic adult IgAV was made. Further laboratory investigations showed a C-reactive protein level of 1 mg/l, erythrocyte sedimentation rate of 7 mm/hour, glomerular filtration rate >90 ml/min, serum IgA of 2.13 g/l (normal level 0.7-4.0 g/l), absence of cryoglobulins, ANA 1:80, anti-dsDNA negative, ENA negative, ANCA negative, normal complement (C3 1.18 g/l, C4 0.15 g/l), HIV negative, hepatitis B negative, hepatitis C negative, EBV and CMV IgM negative. Urine examination disclosed no erythrocytes or proteinuria. An ultrasound of the heart did not show any signs suggestive of endocarditis. Treatment with dapsone 100 mg once daily was started.

Figure 1. *Henoch-Schonlein vasculitis*



Within 24 hours the skin lesions diminished and totally disappeared within a week. During follow-up (12 months), the patient remained asymptomatic with dapsone 50 mg as maintenance therapy.

In conclusion, dapsone was found to be very effective in treating chronic leukocytoclastic vasculitis in our patient with IgAV. The use of dapsone should be advocated in such patients with chronic adult IgAV. The exact mechanism how dapsone works, is poorly understood. Unfortunately, the skin lesions frequently relapse after stopping dapsone and renal involvement does not respond to this treatment.[7-10]

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LETTER TO THE EDITOR

High TPMT activity as a risk factor for severe myelosuppression during thiopurine therapy

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Dear Editor,

The methylating enzyme thiopurine S-methyl transferase (TPMT) plays a crucial role in the metabolism of the thiopurines, azathioprine (AZA) and mercaptopurine (MP). Diminished or absent TPMT activity, leading to elevated levels of the pharmacologically active 6-thioguanine nucleotide (6-TGN), is associated with an increased risk of myelotoxicity. An increased activity of TPMT, which may lead to grossly elevated levels of methylated metabolites (e.g. 6-methylmercaptopurine (6-MMP)), has not been associated with bone marrow suppression.[1] We report a case of an ulcerative colitis (UC) patient who developed a severe pancytopenia due to high TPMT activity.

A male patient, diagnosed with pancolitis ulcerosa, was treated with intravenous cyclosporine, after failure of mesalazine and prednisolone therapy, followed by maintenance treatment with AZA (200 mg). Thiopurine metabolite concentrations were repeatedly low during treatment, without compliance problems. After years, his disease became active again and in attempt to achieve higher 6-TGN levels, AZA was switched to MP (150 mg). After four weeks he developed complaints of gastrointestinal discomfort and general malaise. Laboratory tests showed a severe myelosuppression (leucocyte count 1.0 x 10^9/l and haemoglobin 3.2 mmol/l) and an increased C-reactive protein level of 138 mg/l. Thiopurine metabolites now demonstrated grossly elevated levels of 6-MMP (19,000 mmol/8 x 10^8 RBC) and low 6-TGN concentration (73 mmol/8 x 10^8 RBC). Mercaptopurine therapy was discontinued and he received blood transfusions. Standard viral causes for pancytopenia were ruled out. Endoscopy showed a severely active pancolitis, for which intravenous prednisolone was started and subsequently anti-TNF-α therapy. After ten days, his bone marrow suppression resolved. After discharge, combination therapy with low-dose MP (25 mg) and 100 mg allopurinol was started alongside infliximab. TPMT activity was determined twice, one month after admission (80 nmol/gram protein/hour (reference values: 34-94)) and four months later (121 nmol/g/h). His colitis remained in clinical remission without haematological abnormalities.

In contrast to the well-known association between low TPMT activity and myelosuppression, our case illustrates that high TPMT activity with extremely elevated 6-MMP levels may also induce bone marrow suppression and should be considered a potential risk factor for myelosuppression. 6-Methylmercaptopurine and its ribonucleotides can inhibit the *de novo* purine synthesis leading to a depletion of purines which are essential elements for DNA and RNA formation.[2]

Our case also demonstrates the pitfall of measuring TPMT activity too early after blood transfusions. After one month, the TPMT activity was within normal ranges, while four months later his TPMT activity was found to be elevated. This phenomenon is most likely due to the blood transfusions our patient received during hospitalisation. During the first TPMT measurement donor erythrocytes were still present so the outcome of the test was influenced by the TPMT activity of the donor.

Interestingly, we observed that thiopurine metabolism can change dramatically after switching from AZA to MP with the potential risk of developing severe toxicity, underlining the necessity to strictly monitor patients after modification of thiopurine therapy. Patients displaying grossly elevated 6-MMP and low 6-TGN levels are at risk to develop hepatic transaminitis and may experience therapeutic inefficacy.[3] Combination therapy of allopurinol and low-dose thiopurine can optimise thiopurine therapy in these patients, leading to a steep decrease in 6-MMP levels.[4,5] Our case demonstrates the potential beneficial use of combination therapy as no myelotoxicity reoccurred, indicating that the high 6-MMP levels due to high TPMT activity probably caused the myelosuppression.

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INFORMATION FOR AUTHORS

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2. Kaplan NM. Clinical Hypertension. 7th ed. Baltimore: Williams & Wilkins; 1998.
3. Powell LW, Isselbacher KJ. Hemochromatosis. In: Braunwald E, Fauci AS, Kasper DL, et al., editors. Harrison's Principles of Internal Medicine. 15th edition. New York: McGraw-Hill; 2001. p. 2257-61.

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