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EDITORIAL

Breast cancer genetics: the past, present and future

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Patients and doctors have high expectations when it comes to genetics. The expanding knowledge of the human genome in health and disease should result in early identification of individuals at risk and targets for therapy. To date, different methods have been used to identify genetic variants associated with disease, dependent on availability and technical advancement in basic sciences. In the past, large families with a Mendelian inheritance pattern of a specific trait were studied. Hall *et al.* identified that chromosome 17q21 appeared to be the locus of a gene for inherited susceptibility to breast cancer in families with early-onset disease.[1] Genetic analysis yielded a LOD score (logarithm of the likelihood ratio for linkage) of 5.98 for linkage of breast cancer susceptibility to D17S74 in early-onset families and negative LOD scores in families with late-onset disease.[1] Further investigations demonstrated that this region harboured the well-known BRCA1 gene.[2] Family studies were largely abandoned, because large families which facilitate gene discovery are nowadays scarce.

The next step was to test the association of genetic variants in case-control studies. Technically, this was made possible by the Human Genome Project, which increased our knowledge on 'normal' genetic variation.[3] Initially, only a small set of pre-selected candidate genes could be studied. Each variant needed to be typed by hand, which was a laborious process. These candidate genes were carefully selected based on prior knowledge of the disease.

In the current issue of the Netherlands Journal of Medicine, Li *et al.* publish a systemic review and meta-analysis on such a candidate gene for breast cancer: CASP8 -652.[4] In the early 2000s many studies showed that caspases are at the crossroads of immune cell life and death, and their aberrant expressions or activities are associated with many pathological conditions, including cancer. Therefore, variation in this gene was studied in 1K cases and controls.[5] Even though the initial study showed a clear association, following individual studies showed conflicting results. Li *et al.* test the hypothesis that deletions in CASp9 are associated with a reduced risk of breast cancer in the largest study to date: 13,220 cases and 13,750 controls. After combining the data, a specific polymorphism CASP* -652 6N del is associated with a reduced risk (homozygous carriers; R=0.78, 95% CI 0.63-0.95; dominant OR=0.93, 95 % CI 0.88-0.99). They rightfully conclude that large sample studies using standardised unbiased genotyping methods, in homogenous breast cancer patients and well-matched controls, are needed to ultimately lead to a better understanding of the association of CASP8-652N del and breast cancer.

In the present, thanks to technical advancement and the development of DNA chips containing hundreds of thousands of genetic variants, the standards for gene discovery have evolved from candidate gene approaches to unbiased whole genome approaches in the Genome-Wide Association Studies (GWAS). A recent study of 10K breast cancer cases and 12K controls followed by a replication study of 43K cases and 42K controls identified 41 loci associated with breast cancer.[6] In contrast to Li *et al.* the latest GWAS unfortunately no longer demonstrates a clear association between variation in CASP8 and breast cancer. The GWAS studies have the power to detect an association with common variants and disease. The majority of these variants have a small effect on the disease phenotype. Individually, they can therefore not yet be applied to predict breast cancer. However, incorporating these loci into risk models is expected to improve disease prediction. The latest developments are sequencing all protein coding regions of large cohorts of individuals in the hope to identify rare variants with large effects. Incorporating these into models will probably improve them. One should bear in mind that association does not imply causality. In depth sequencing of regions associated with disease should result in the identification of causal variants.

In the future, functional studies are needed to understand, if and through which pathophysiological mechanisms the identified genetic variation result in disease.

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REVIEW

High-sensitivity troponin after running – a systematic review

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ABSTRACT

A systematic review was carried out to study the pattern of high-sensitivity cardiac troponin release after running (search performed on PubMed, ISI Web of Knowledge and Scopus databases). A total of ten reports were identified as meeting the pre-specified criteria (eight using high-sensitivity troponin T and two using high-sensitivity troponin I). The papers were published between 2009 and 2013, amounting to a total of 479 participants under study. Eight reports provided data comparing post-running troponin levels with the 99th percentile reference value. A total number of 296 participants, out of 424, showed post-running high-sensitivity troponin values higher than the 99th percentile reference value (69.8%). In conclusion, using high-sensitivity cardiac troponin assays, studies have shown that elevated post-running values are seen in more than two-thirds of runners. Whether troponin release in this setting represents a fully reversible phenomenon is currently unknown; the effects of strenuous running on long-term health are also uncertain.

KEYWORDS

Running, high-sensitivity troponin

INTRODUCTION

Cardiac troponin has been shown to act as a most useful biomarker in the context of acute myocardial infarction, and is usually believed to act as a marker of necrosis in this setting.[1-3]

However, research carried out in other settings has shown that increased plasma levels of cardiac troponin are a relatively frequent finding, including in patients with chest pain of other causes, sepsis, pulmonary embolism, aortic valve disease, and heart failure.[3]

Running is a normal physiological phenomenon in humans. Studies carried out since 1987 have shown that prolonged and/or strenuous running is associated to increased plasma levels of cardiac troponin.[4]

Previous systematic reviews have been published on this topic.[5,6] However, technical improvements in laboratory techniques have led to the current availability of high-sensitivity assays – usually believed to be more reliable in the evaluation of plasma cardiac troponins, namely in what concerns values closer to the detection limit of the assay.[7]

The aim of this systematic review was to present the current state of the art concerning cardiac troponin level changes associated with running, including long distance or strenuous running, as supported by studies carried out using the high-sensitivity assays. It is, to our knowledge, the first systematic review to consider exclusively high-sensitivity troponin assays in the evaluation of this interesting and common phenomenon.

METHODS

Search strategy

The study started with a search on three databases, Medline (PubMed), ISI Web of Knowledge and Scopus, using the query "troponin" + "running". In PubMed, the additional keyword "marathon" was also used ("troponin AND ((running) OR (marathon))").

The search took place between April and May 2013, and no articles were excluded based on publication date. The aim of our search was to identify studies evaluating the levels of cardiac troponins (either T or I) using high-sensitivity assays, as defined by the authors, in association with a period of running (regardless of intensity, duration of exercise or length, or of previous physical exercise practice).

The query resulted in 157 articles on the PubMed database, 259 on ISI Web of Knowledge and 181 on Scopus. No additional studies were found after searching the references of previous review articles.

Inclusion criteria

Only prospective observational human studies were included. It was mandatory for the studies to evaluate the levels of cardiac troponins before and after the race, and only studies using high-sensitivity troponin assays (as defined by the authors in the title and/or abstract) met the inclusion criteria.

Exclusion criteria

Articles in which the subjects were selected because they had a specific pathology, case reports and articles that did not use high-sensitivity troponin assays were excluded. Articles written in languages other than English, as well as mechanistic and animal studies, were also excluded. Studies containing less than ten subjects were excluded too. Studies evaluating exercise but not specifically running, such as cycling or triathlon, were excluded because, although of importance, they were outside the scope of the present report.

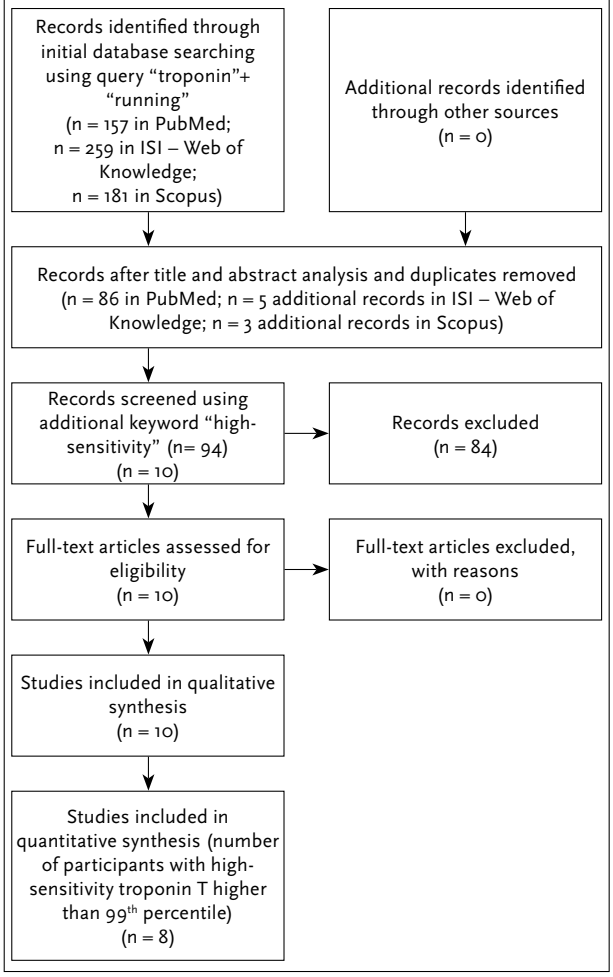
Summary measure

The primary summary measure in the quantitative analysis was the determination of the number of participants with high-sensitivity troponin values greater than the 99th percentile after the race. The number of participants in some studies were calculated from the published value corresponding to the percentage.

Quality assessment of studies and data extraction

Study quality and eligibility were individually assessed by four investigators. Different opinions regarding the relevance of articles were solved by consensus between the authors.

Figure 1. *Flowchart showing the literature search method (n = number)*



RESULTS

From title and abstract analysis, ten articles were included that met the pre-specified criteria, and this set of articles was analysed by the authors.[8-17] A flowchart showing the literature search method, as well as the resulting number of articles selected, is displayed in *figure 1*. The total of ten articles that were selected for qualitative review were published between 2009 and 2013, amounting to a total of 479 subjects in which high-sensitivity cardiac troponin was assessed.

Table 1 presents the main characteristics of the subjects involved in each study. For the quantitative synthesis regarding the percentage of participants with high-sensitivity troponin greater than the 99th percentile (*table 2*), a total of eight trials were assessed, amounting to 424 runners (381 males), of which 296 had high-sensitivity troponin levels greater than the 99th percentile (69.8% of the total). In this synthesis, 392 runners had high-sensitivity troponin T assessed, and 273 of those patients (69.6% of the total) had troponin levels greater than the 99th percentile, when evaluated after the race (immediately or within six hours after the race). Thirty-two runners had high-sensitivity troponin I assessed, and 23 of those (71.9% of the total) had levels greater than the 99th percentile, when evaluated after the race (within six hours of completion).

Table 3 presents an overview of the main conclusions in the studies included in the qualitative review.

DISCUSSION

In the present report, a systematic review was undertaken to look at changes in plasma troponin levels after running – an important physiological activity of the human body. Only reports dealing with high-sensitivity troponin were chosen, since these new assays are usually believed to be more reliable in the evaluation of plasma cardiac troponins,

Table 1. *Overview of trials meeting the pre-specified criteria*

Study (year)	N	Male/ female ratio	Mean age (years)	Type of race (length in km)	Biomarkers assessed	Timing of sample collection
Mingels *et al.* (2009)[8]	85	70/15	47	Marathon (42.2 km)	TnI TnT HSTnT CK Albumin	0-2 hours before the race <1 h after the race
Giannitsis *et al.* (2009)[9]	10	10/0	52	Ultramarathon (216 km)	HSTnT NT-proBNP	Baseline After first half marathon, full, double and quadruple marathon Shortly after the finish
Mingels *et al.* (2010)[10]	43; 38; 10; 85	24/19; 31/7; 8/2; 70/15	45; 47; 43; 47	5, 15, 21, 42 km	HSTnT NT-proBNP Albumin	0-2 hours before the race <1 h after the race
Saravia *et al.* (2010)[11]	78	78/0	56 (median)	Marathon (presumably, 42.2 km)	TnT HSTnT Leukocytes CRP IL-6 NT-proBNP	Before the race <20 minutes after finishing the race Nearly two weeks after the race
Scherr *et al.* (2011)[12]	102	102/0	42	Marathon (42.195 km)	HSTnT NT-proBNP h-FABP TNF-α IL-6 IL-10 Hs-CRP Cystatin C Hg Haematocrit Albumin	During the week before the race Within 1 h after the race 24 hours after the race 72 hours after the race
Lippi *et al.* (2012)[13]	15	15/0	41	Ultramarathon (60 km)	TnI HSTnI	Before the race (20 minutes before warm-up) Within 10 minutes after the race
Tian *et al.* (2012)[14]	13+13	26/0	14.1; 24	Constant load treadmill run for 90 minutes	HSTnT NT-proBNP Hg Haematocrit	Pre-exercise Immediately post-exercise 1, 2, 3, 4, 5, 6, and 24 hours post-exercise
Lippi *et al.* (2012)[15]	17	17/0	47	Half marathon (21 km)	TnI HSTnI	Before the race (30 minutes before warm-up) Immediately after the race, and at 3, 6 and 24 hours after the race
Wilhelm *et al.* (2012)[16]	10	10/0	34.9	Mountain marathon	HSTnT Pro-ANP HS-CRP IL-6 TNF-α Leukocytes Hg Haematocrit Plasmatic sodium	Baseline Within 15 minutes after the race 1 and 8 days after the race
Baker *et al.* (2013)[17]	45	-	-	Marathon (41.84 km)	TnI TnT HSTnT BNP	Before the race Within 15 minutes after the race

n = number of runners; BNP = brain natriuretic peptide; CK = creatine kinase; h-FABP = heart-type fatty acid binding protein; Hg = haemoglobin; HS-CRP = high-sensitivity C reactive protein; IL-6 = interleukin 6; NT-proBNP = N-terminal pro-hormone of brain natriuretic peptide; pro-ANP = pro-atrial natriuretic type; TNF-α = tumour necrosis factor α; TnI = cardiac troponin I; TnT = cardiac troponin T; HSTnI = high-sensitivity troponin I; HSTnT = high-sensitivity troponin T.

namely in what concerns values closer to the detection limit of the assay.[7]

The main finding was that a considerable percentage of the study participants were shown to have increases in plasma troponin values, not only when compared with baseline values, but also when compared with the 99th percentile value for the biomarker under study.

According to one point of view,[18] elevated cardiac troponin plasma values would always correspond to cardiomyocyte necrosis – a theory that, in the case of strenuous running, and in the light of the present report, would correspond to a majority of runners having undergone cardiac cellular necrosis – although, presumably, of low grade. This theory, however, and in the case of running, is currently not

Table 2. *Number of participants with high-sensitivity cardiac troponin values, measured up to six hours after the race, higher than the 99th percentile, presented as a fraction of the total number of participants (number of participants in some studies were calculated from the published value corresponding to the percentage)*

Mingels *et al.* (2009)[8]	73/85
Giannitsis *et al.* (2009)[9]	4/10
Mingels *et al.* (2010)[10]	0/ 43 (5 km) 5/ 38 (15 km) 4/10 (21 km)
Saravia *et al.* (2010)[11]	73/78
Scherr *et al.* (2011)[12]	91/102
Lippi *et al.* (2012)[13]	12/15
Tian *et al.* (2012)[14]	12/13 (adolescents) 11/13 (adults)
Lippi *et al.* (2012)[15]	11/17
Total	296/424 (69.8%)

Table 3. *Major findings concerning high-sensitivity cardiac troponin, in ten articles selected in the course of the systematic review*

Study (year)	Major findings concerning high-sensitivity troponin
Mingels *et al.* (2009)[8]	Reference values of high-sensitivity troponin T higher for males 100% of runners with increase in troponin I and high-sensitivity troponin T after the race
Giannitsis *et al.* (2009)[9]	10 cases with a rise of high-sensitivity troponin T 1 case with early rise of high-sensitivity troponin T, followed by decrease to baseline value Heterogenous behaviour of troponin concentration in different runners
Mingels *et al.* (2010)[10]	Troponin concentrations significantly higher with increasing running distance except in the 5 km race group
Saravia et al. (2010)[11]	High-sensitivity troponin T increased significantly after the race 2 weeks after the race troponin levels were comparable with baseline levels Larger number of participants with increased troponin levels with high-sensitivity assay, when compared with older types of assays
Scherr *et al.* (2011)[12]	Increase above threshold was observable in 89% of participants immediately after the race, in 27%, 24 h after the race, and in 4%, 72 h after the race
Lippi *et al.* (2012)[13]	80% of participants with high-sensitivity troponin I >99th percentile, after the race
Tian *et al.* (2012)[14]	Post-exercise high-sensitivity troponin T elevation occurred in all runners, peaked 3-4 hours post-exercise, and the peak concentration was higher in adolescents than adults During the recovery phase, high-sensitivity troponin T significantly higher in adolescents than in adults
Lippi *et al.* (2012)[15]	High-sensitivity troponin I significantly increased from the mean baseline value of 2.9-4.8 ng/l after the run, 9.0 ng/l at 3 hours, 12.3 ng/l at 6 hours, and 4.5 ng/l at 24 hours 65% of participants with high-sensitivity troponin I >99th percentile 6 hours after the race
Wilhelm *et al.* (2012)[16]	High-sensitivity troponin T increased significantly after the race and returned to baseline during follow-up
Baker *et* al. (2013)[17]	Mean high-sensitivity troponin T increased after the race from 3-47 ng/l 100% runners with detectable levels of high-sensitivity troponin T

supported by empirical data – since pathological studies indicating the presence of necrosis were not found in the literature.

In studies assessing both troponin release and cardiac magnetic resonance imaging, no detectable myocardial necrosis was observed after marathon running.[19-22] A half marathon was associated to an increase in right ventricular end-diastolic volume, with a reduction in the right ventricular ejection fraction.[23]

Breuckmann *et al.* described late gadolinium enhancement in 12 out of 102 healthy marathon runners, a value which was compared with four cases in a control population, yielding a p value of 0.077.[24] The study did not involve either troponin measurements or pre-marathon cardiac studies,[24] and was of an observational nature, thus unable to establish causality. Cyclists from the Tour de France were shown to have an increased average longevity, when compared with the general population.[25]

The results presented in the present report point in the direction that cardiac troponin release associated with running, including marathon running, occurs in more than two-thirds of the participants studied. Previous systematic reviews looking at data obtained prior to the development of high-sensitivity troponin testing have been published. Shave *et al.* reported troponin T to exceed assay detection limit in 52% of participants in running events.[6] Regwan *et al.* reported an incidence of post-marathon troponin elevation of 51%.[5] Data obtained with high-sensitivity troponin testing, in summary, shows troponin release after running to be a more generalised phenomenon than previously thought.

Cardiomyocyte necrosis could be the mechanism behind troponin release in this setting – as stated above, an unsubstantiated allegation, in as much as no histological proof for this phenomenon has been put forward. Even the data presented by Breuckmann *et al.*, which could have causes other than running, show a strikingly low value of 12% for the presence of late gadolinium enhancement,[24] when compared with a 69.8% mean value of running participants with troponin release, now reported. The time course of troponin release in this setting – with a relatively rapid decrease in plasma cardiac troponin values after peak values are reached – would also argue in favour of a reversible phenomenon. Cardiac strain[26,27] could be an explanation for the phenomenon of troponin release after strenuous running, in good agreement with data obtained under experimental conditions, perhaps implicating integrin stimulation as a mechanism behind troponin release.[28]

Study limitations

Given the heterogeneous nature of the data (physical pre-conditioning of the subjects, intensity, length and duration of the running period) and the possible presence of confounding factors (such as associated pathologies, especially cardiovascular disease and its protean manifestations) it is difficult to regard the data presented above as a set obtained in a homogeneous population. Many of the studies only included male participants, and the pattern of troponin release after running in the female gender should be further clarified in future studies. It is also clear (as shown in *table 1*) that most studies assessed high-sensitivity troponin T, whereas high-sensitivity troponin I was assessed in only two studies.[13,15] As Lippi *et al.* suggest[13] the data obtained with the two biomarkers seem to be comparable, but more data on this matter would be desirable. On the other hand, while the value corresponding to the 99th percentile was the same regarding the high-sensitivity troponin I assay in both trials (i.e. 8.6 ng/l), this was not the case for the high-sensitivity troponin T assays, different values being used in this latter case.

Further studies are needed to better establish the clinical significance, if any, and the long-term prognosis associated with high-sensitivity troponin elevations in this setting.

In conclusion, in the present systematic review, using data obtained with high-sensitivity cardiac troponin assays, elevations of cardiac troponin plasma levels were shown to exist in more than two-thirds of the participants studied, pointing to a more generalised phenomenon than previously thought.

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REVIEW

CASP8 -652 6N del polymorphism and breast cancer risk: a systematic review and meta-analysis

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ABSTRACT

Purpose: Many studies have investigated the association between CASP8 -652 6N del polymorphism and the risk of breast cancer, but the result is still unclear owing to the obvious inconsistence among those studies. This study aims to quantify the strength of association between CASP8 -652 6N del polymorphism and risk of breast cancer.

Methods: We searched the electronic MEDLINE database for studies relating to the association between CASP8 -652 6N del polymorphism and risk of breast cancer. We estimated summary odds ratios (ORs) with their 95% confidence intervals (95% CIs) to assess the association. Ten case-control studies with 13,220 cases and 13,750 controls were included into this meta-analysis.

Results: Meta-analysis of a total of ten studies showed that reduced breast cancer risk was associated with CASP8 -652 6N del polymorphism (homozygous: OR=0.85, 95% CI 0.93-0.98). After adjustment for heterogeneity, meta-analysis showed that reduced breast cancer risk was also associated with CASP8 -652 6N del polymorphism (homozygous: OR=0.78, 95% CI 0.63-0.95, dominant: OR=0.93, 95% CI 0.88-0.99). For Caucasians, CASP8-652 6N del was associated with reduced breast cancer risk at a borderline level (homozygous: OR=0.94, 95% CI 0.86-1.02, heterozygous: OR=0.96, 95% CI 0.90-1.03, recessive: OR=0.96, 95% CI 0.90-1.03, dominant: OR=0.94, 95% CI 0.88-1.01). No evidence of publication bias was observed.

Conclusion: Meta-analyses of the available data suggest that CASP8 -652 6N del polymorphism is associated with reduced breast cancer risk.

KEYWORDS

CASP8 -652 6N del polymorphism, breast cancer risk, meta-analysis

INTRODUCTION

Apoptosis, also called programmed cell death, is important to maintain internal homeostasis by removing irreparable damaged cells. Defects in apoptosis machinery may lead to cancer.[1] The Caspase-8 (CASP8) protein regulates apoptosis, and it stimulates cell proliferation, malignant transformation and tumour progression as a result of its dysfunction or reduced activity.[2] CASP8 is encoded by the CASP8 gene. The human CASP8 gene contains at least 11 exons spanning ~30 kb on the highly polymorphic chromosome 2q33-34.[3] Several studies have confirmed that in addition to rare mutations, a few common variants of the CASP8 gene disrupt the apoptotic mechanism and thus impact the risk of developing various types of cancer, including breast cancer,[4] prostate cancer[5] and several other cancers.[6] Previous studies have largely focused on two variants of the CASP8 gene: D302H (rs1045485) and -652 6N del (rs3834129). Although the results of studies on the D302H variant have been generally consistent, conclusions on the -652 6N del variant remain inconsistent and inconclusive; some studies have demonstrated reduced susceptibility,[7] whereas other studies did not detect any association.[8-10] Zhang *et al.*[11] found that CASP8 -652 6N del polymorphism was not associated with breast cancer risk. However, their meta-analysis was only a small part of their original paper. When they performed the meta-analysis, the pooled sample size was relatively small and not enough information was available for more exhaustive subgroup analysis. Since then, several additional studies about this polymorphism and breast cancer risk, with large sample sizes, have been reported, which would greatly improve the power of the meta-analysis of this polymorphism. Subgroup analyses performed by ethnicity were also possible now. Therefore, we performed an updated meta-analysis on all the available case-control studies to access the breast cancer risk with CASP8 -652 6N del.

METHODS

Identification and eligibility of relevant studies

We searched for relevant papers published before 24 May 2013 in the English literature by using the electronic MEDLINE database with the following terms "CASP8", "caspase 8", "-652 6N del", "rs3834129", "breast cancer", "polymorphism" and "variant". References of the retrieved articles were also screened for original studies. We included all the case-control studies and cohort studies that investigated the association between CASP8-652 6N del polymorphisms and breast cancer risk with genotyping data. Abstracts, unpublished reports and articles not written in the English language were not considered. Additionally, when a case-control study was included by more than one article using the same case series, we selected the study that included the largest number of individuals.

Data extraction

We extracted the following information from each manuscript: author, year of publication, country of origin, ethnicity and genotyping information. For studies including subjects of different ethnicities, data were extracted separately and categorised as Asians, Caucasians and mixed-race individuals.

Statistics

Based on the genotype frequencies in cases and controls, crude odds ratios (ORs) as well as their standard errors (SEs) were calculated. Pooled ORs were calculated for the homozygous genetic model, heterozygous genetic model, dominant genetic model and recessive genetic model, respectively. The fixed effects model (Mantel-Haenszel method) as well as the random effects (DerSimonian Laird) model were used to calculate the pooled OR. Between-study heterogeneity and between-study inconsistency were assessed by using Cochran Q statistic and by estimating I^2, respectively.[12] Heterogeneity was considered to be significant when $p<0.10$ and $I^2>50\%$. When significant heterogeneity was detected, the random effects model was chosen; nevertheless, the fixed effects estimates are also secondarily reported as an alternative approach. To study the source of between-study heterogeneity, the Galbraith plot was used to spot the outliers as possible major sources of between-study heterogeneity.[13] Evidence of publication bias was determined using Begg's[14] and Egger's[15] formal statistical test and by visual inspection of the funnel plot. All statistical tests were conducted with Review Manager downloaded from the Cochrane Collaboration website (Version 5.1). A p value of 0.05 for any test or model was considered to be statistically significant.

RESULTS

Study characteristics

A total of six publications met the inclusion criteria.[7,10,16-19] In two of these studies, the ORs were presented separately according to the different subgroups.[17,18] Therefore, each group in one publication was considered separately for subgroup analysis. Hence, a total of ten studies including 13,220 cases and 13,750 controls were used in the meta-analysis. *Table 1* lists the studies identified and their main characteristics. Among these studies, there were seven studies of Caucasians, one study of Asians and two studies of mixed populations. Almost all of the cases were pathologically confirmed. Controls were mainly healthy populations and matched for age.

Main results

Table 2 lists the main results of this meta-analysis. There was obvious between-study heterogeneity among these ten studies (homozygous: $I^2=67.3\%$, heterozygous: $I^2=64\%$, recessive: $I^2=63.9\%$, dominant: $I^2=71\%$), thus the random effects model was used to pool data. Meta-analysis showed that reduced breast cancer risk was associated with CASP8 -652 6N del polymorphism (homozygous: OR=0.85, 95% CI 0.93-0.98). After adjustment for heterogeneity by the Galbraith plot, there was no between-study heterogeneity among the remaining studies, thus the fixed effects model was used to pool the ORs. Meta-analysis showed that reduced breast cancer risk was also associated with CASP8 -652 6N del polymorphism (homozygous: OR=0.78, 95%

Table 1. *Main characteristics of all the studies included in the meta-analysis*

Study	Country	Ethnicity	Case	Control	Genotype method
Sun 2007[7]	China	Asian	1119	1004	PCR-RFLP
Cybulski 2008[16]	Poland	Caucasian	618	965	PCR-RFLP
Frank 2008[17]	Germany	Caucasian	1110	1108	Fluorescent analysis
Frank 2008[17]	UK	Caucasian	1212	1184	Fluorescent analysis
Frank 2008[17]	Germany	Caucasian	1143	1155	Fluorescent analysis
Frank 2008[17]	Germany	Caucasian	4470	4560	Fluorescent analysis
Haiman 2008[18]	USA	Mixed	2029	2245	TaqMan
Haiman 2008[18]	USA	Mixed	703	920	TaqMan
De Vecchi 2009[10]	Italy	Caucasian	580	406	PCR-RFLP
Hashemi 2012[19]	Iran	Caucasian	236	203	PCR premix

Table 2. *Main results of pooled ORs in the meta-analysis*

	Studies	OR (95% CI)	P_{OR}	Model	I^2 (%)	P_H
Homozygous	10	0.85 (0.73-0.98)	0.028	Random	67.3	0.001
Homozygous (adjustment for heterogeneity)	2	0.78 (0.63-0.95)	0.003	Fixed	0	0.915
Heterozygous	10	0.93 (0.84-1.02)	0.052	Random	64	0.003
Heterozygous (adjustment for heterogeneity)	8	0.94 (0.89-1.00)	0.067	Fixed	0	0.951
Recessive	10	0.89 (0.78-1.00)	0.053	Random	63.9	0.003
Recessive (adjustment for heterogeneity)	8	0.96 (0.90-1.03)	0.246	Fixed	32.1	0.172
Dominant	10	0.91 (0.81-1.01)	0.074	Random	71	0
Dominant (adjustment for heterogeneity)	8	0.93 (0.88-0.99)	0.022	Fixed	0	0.681

Table 3. *Main results of pooled ORs in the meta-analysis of the Caucasian populations*

Studies with poor design	Studies	OR (95% CI)	P_{OR}	Model	I^2 (%)	P_H
Studies with poor design	7	0.86 (0.74-1.01)	0.062	Random	60.4	0.019
Studies with poor design	6	0.94 (0.86-1.02)	0.116	Fixed	20.6	0.278
Heterozygous	7	0.39 (0.33-0.47)	0	Random	71.6	0.002
Heterozygous (adjustment for heterogeneity)	6	0.96 (0.90-1.03)	0.277	Fixed	0	0.967
Recessive	7	0.89 (0.77-1.02)	0.091	Random	65	0.009
Recessive (adjustment for heterogeneity)	6	0.96 (0.90-1.03)	0.244	Fixed	42.3	0.123
Dominant	7	0.94(0.88-1.01)	0.089	Random	0	0.623

CI 0.63-0.95, dominant: OR =0.93, 95% CI 0.88-0.99) (*figures 1 and 2*).

In the subgroup analysis by ethnicity, CASP8 -652 6N del polymorphism was associated with reduced breast cancer risk at a borderline level after adjustment for heterogeneity (homozygous: OR=0.94, 95% CI 0.86-1.02, heterozygous: OR=0.96, 95% CI 0.90-1.03, recessive: OR=0.96, 95% CI 0.90-1.03, dominant: OR=0.94, 95% CI 0.88-1.01, *table 3*). The borderline character of the association may be due to relatively inadequate overall power.

Publication bias

Begg's funnel and Egger's test were performed to access the publication bias in this meta-analysis. The shape of the funnel plots did not reveal obvious evidence of asymmetry, and the p value of Egger's test was >0.05 (homozygous: p=0.79, heterozygous: p=0.788, recessive: p=0.245, dominant: p=0.531), providing statistical evidence of funnel plot symmetry (*figure 3*). Thus, the results above suggest that publication bias was not evident in this meta-analysis.

DISCUSSION

It is well recognised that there is individual susceptibility to the same kind of cancer even with the same environmental exposure. Host factors, including polymorphisms of genes involved in carcinogenesis, may have accounted for this difference. Therefore, genetic susceptibility to cancer has been a research focus in the scientific community; CASP8, encoded by the CASP8 gene, has a central function in apoptotic pathways and changes in the genetically determined structure of this enzyme can influence the rate of apoptosis. More specifically, a six nucleotide deletion polymorphism (-652 6N del) has been identified in the promoter region of the CASP8 gene and is associated with decreased RNA expression in lymphocytes due to the altering of an Sp1 binding site.[20] This variant has been found to decrease CASP8 activity and apoptotic reactivity of T lymphocytes through the cancer cell *ex vivo* model. Recently, due to the functional significance of the CASP8 -652 6N del variant, genetic variants of the CASP8 gene in the aetiology of several cancers have drawn increasing attention. A growing number of studies have suggested that -652 6N del in the promoter region of the CASP8 gene was associated with decreased breast cancer risk. However, the results are inconclusive. To better understand the association between this polymorphism and breast cancer risk, a pooled analysis with a large sample and heterogeneity explored is necessary.

Thus, we performed this meta-analysis by critically reviewing ten individual case-control studies with a total of 13,220 breast cancer cases and 13,750 controls. Meta-analysis of the ten studies showed that reduced breast cancer risk was associated with CASP8 -652 6N del polymorphism (homozygous: OR=0.85, 95% CI 0.93-0.98). Heterogeneity is a potential problem when interpreting the results of all meta-analyses, and finding the sources of heterogeneity is

Figure 1. *Forest plots showing associations between CASP8 -652 6N del polymorphism and risk of breast cancer. A) Analysis of all the included studies. B) Analysis of all the studies after adjustment for heterogeneity*

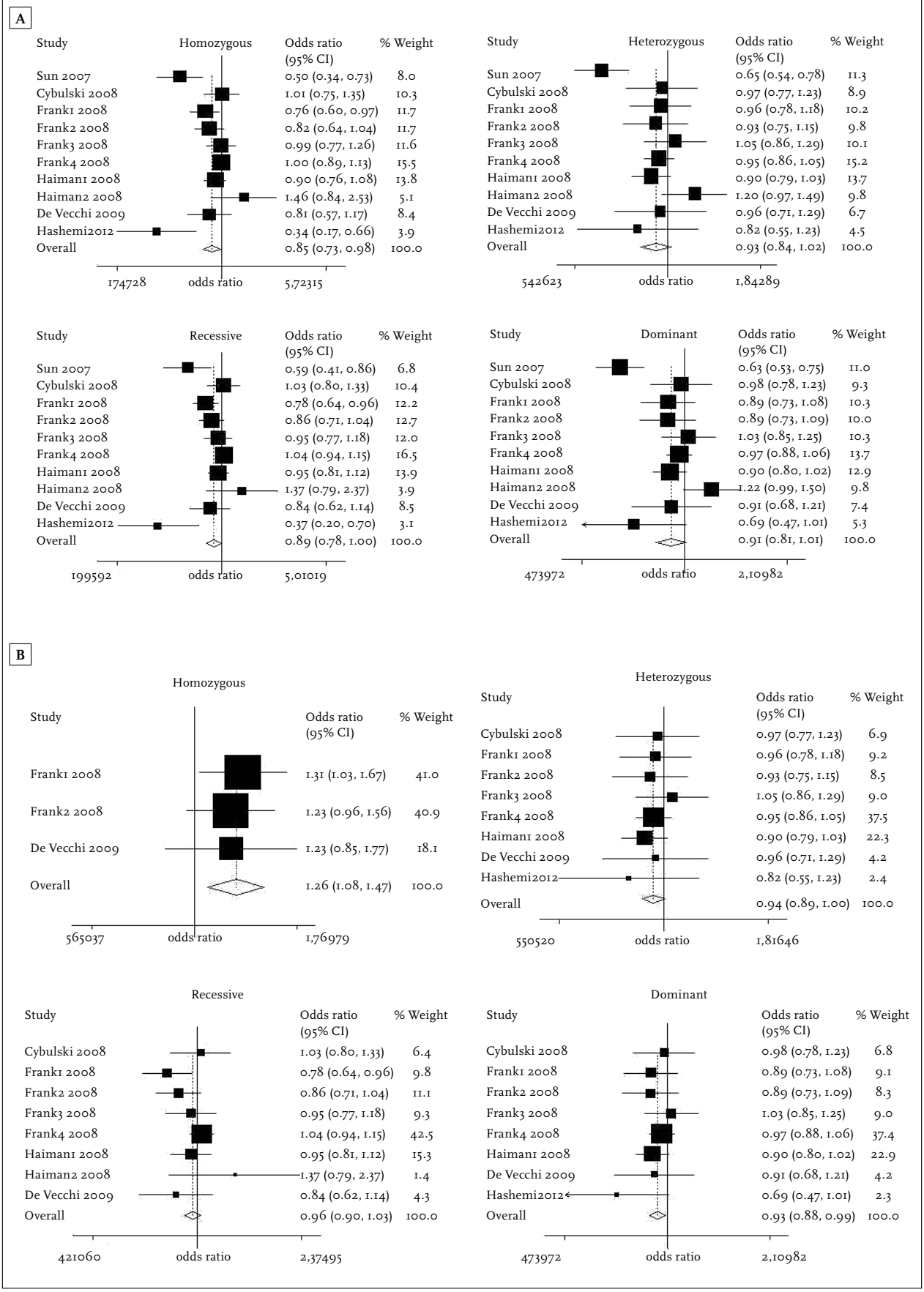


Figure 2. *Galbraith plots of association between CASP8 -652 6N del polymorphism and breast cancer risk. A) Galbraith plot of meta-analysis of all the included studies. B) Galbraith plot of meta-analysis of the Caucasian studies*

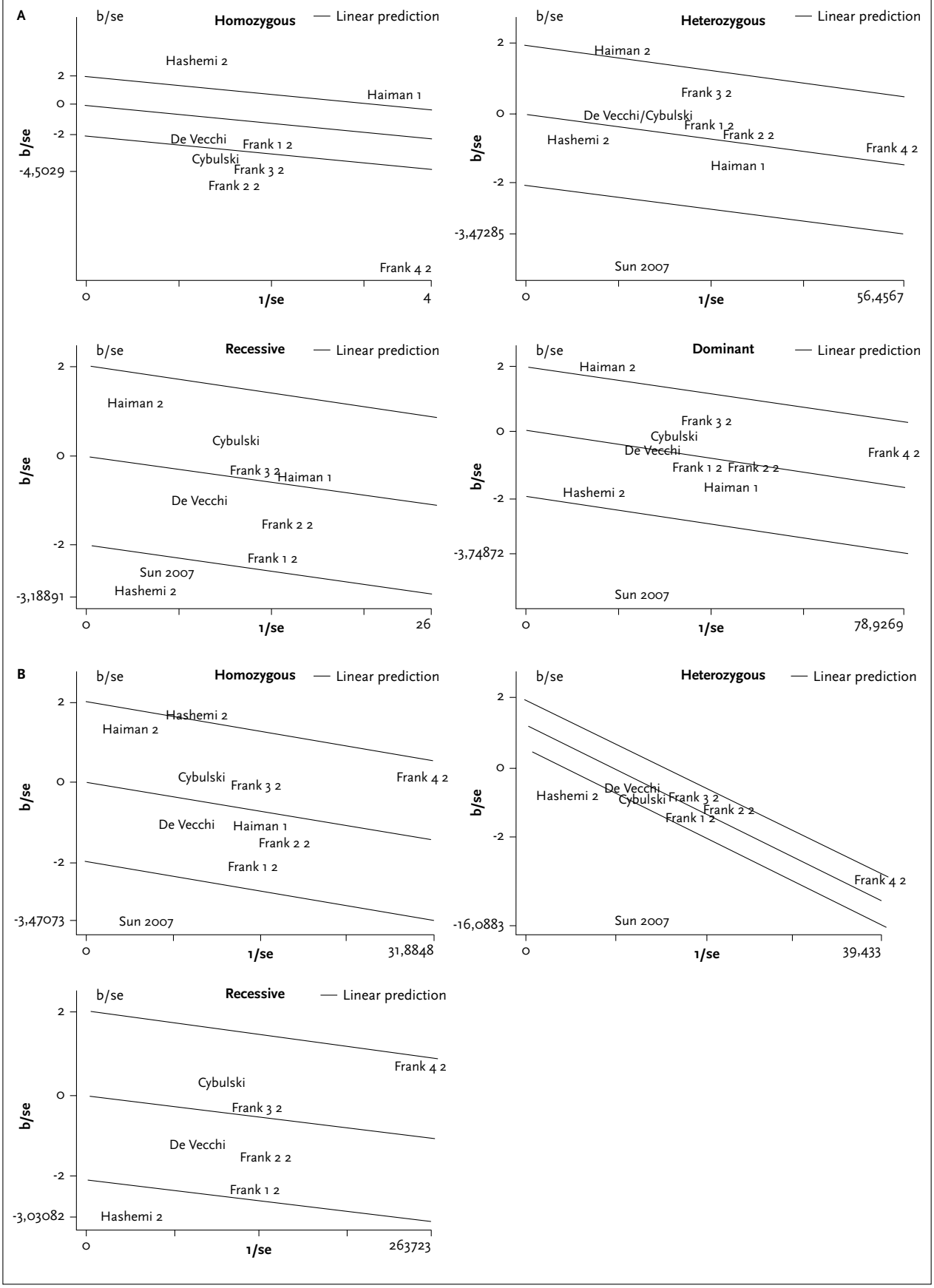
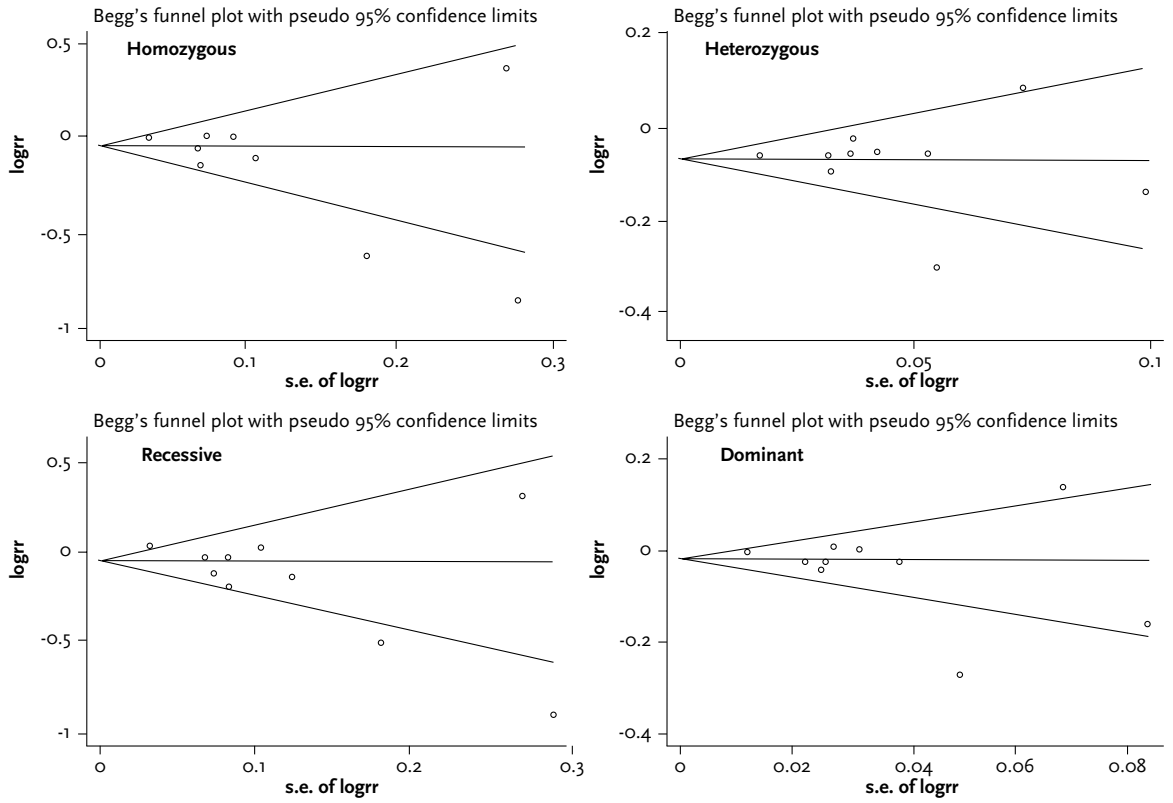


Figure 3. *Begg's funnel plots for assessing the risk of publication bias*



one of the most important goals of meta-analysis.[21] In this present meta-analysis, we found obvious heterogeneity in the meta-analysis of the ten studies (homozygous: I^2=67.3%, heterozygous: I^2=64%, recessive: I^2=63.9%, dominant: I^2=71%). Heterogeneity may also come from the studies with a poor design, because these studies usually do not exclude possible factors that may bias the estimate of the real effects, and may result in incorrect conclusions.[22] Thus, the Galbraith plot was used to spot the outliers as possible studies with low quality design. After excluding those studies, the between-study heterogeneity decreased and there was no obvious heterogeneity among the remaining studies, which further suggested the heterogeneity might come from those studies. After adjustment for heterogeneity, meta-analysis showed that reduced breast cancer risk was also associated with CASP8 -652 6N del polymorphism (homozygous: OR=0.78, 95% CI 0.63-0.95, dominant: OR=0.93, 95% CI 0.88-0.99). Thus, meta-analyses of the available data supported an association between the CASP8 -652 6N del polymorphism and reduced breast cancer risk. So, the outcomes above provide further evidence for the association between CASP8 -652 6N del genotype and decreased risk of breast cancer.

Some limitations of this meta-analysis should be acknowledged. First, in the subgroup analyses, the number of Asians was relatively small, not having enough statistical power to explore the real association. Additionally, no data were available about Africans. Second, our results were based on unadjusted estimates, while a more precise analysis could have been conducted if individual data had been available, which would allow for the adjustment by other co-variants including age, ethnicity, menopausal status, smoking status, drinking status, obesity, environmental factors, and other lifestyle.

In conclusion, this meta-analysis suggests that the CASP8 -652 6N del polymorphism is associated with decreased breast cancer risk. However, it is necessary to conduct large sample studies using standardised unbiased genotyping methods, homogeneous breast cancer patients and well-matched controls. Moreover, gene-gene and gene-environment interactions should also be considered in the analysis.[23] Such studies taking these factors into account may eventually lead to our better, comprehensive understanding of the association between the CASP8 -652 6N del polymorphism and breast cancer risk.

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REVIEW

Venous thromboembolism during hip plaster cast immobilisation: Review of the literature

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ABSTRACT

Introduction: There is a paucity of data regarding the risk of deep vein thrombosis during hip plaster cast immobilisation. The purpose of this article was to review the available evidence regarding the incidence of symptomatic venous thromboembolism (VTE) during hip plaster cast immobilisation.

Methods and Materials: All papers describing hip plaster cast immobilisation published in the English literature retrieved from PubMed, EMBASE and the Cochrane database were reviewed. Articles regarding children, hip dysplasia, congenital hip dislocation and Legg-Calvé-Perthes were excluded. A total of three papers were available for analysis. We also describe a case of pulmonary embolism during hip cast immobilisation.

Results: The overall incidence of symptomatic VTE during hip plaster cast immobilisation was 0% in 343 patients. The incidence of symptomatic VTE in hip cast brace was 2.3% (range 0-3%).

Discussion: Our systematic review of the literature showed a paucity of data regarding the incidence of VTE during hip plaster cast immobilisation. We describe the first case of pulmonary embolism during hip plaster cast immobilisation. We recommend that patients who are fitted with a hip plaster cast should be routinely screened for additional risk factors. When risk factors are present, patients should be considered for pharmacological thromboprophylaxis.

KEYWORDS

Plaster cast, pulmonary embolism, spondylodesis, venous thromboembolism, systematic review

What was known on this topic?

Immobilisation is a major risk factor for VTE. A meta-analysis on lower leg plaster cast immobilisation showed a highly significant and clinically relevant reduction in asymptomatic events with LMWH prophylaxis compared with placebo or untreated controls.

What does this add?

A systematic paper review on the incidence of symptomatic VTE during hip plaster cast immobilisation is presented. The first case of pulmonary embolism during hip plaster cast immobilisation is described. We recommend that patients who are fitted with a hip plaster cast should be routinely screened for additional VTE risk factors. When risk factors are present, patients should be considered for pharmacological thromboprophylaxis.

INTRODUCTION

There is a paucity of data regarding the risk of deep vein thrombosis during hip plaster cast immobilisation. The purpose of this article was to review the available evidence regarding incidence of symptomatic venous thromboembolism (VTE) during hip plaster cast immobilisation.

Furthermore, we describe a case of pulmonary embolism in a hip plaster cast. To our knowledge, this has not been documented before. Clinicians should be aware of the risk of venous thromboembolism (VTE) when treating a patient with plaster cast immobilisation. A meta-analysis

reported a mean rate of VTE during *lower leg* plaster cast immobilisation without thromboprophylaxis of 17.1%.[1]

MATERIAL AND METHODS

A systemic search strategy was used to identify all papers describing symptomatic venous thromboembolism (deep venous thrombosis and pulmonary embolism) during hip cast immobilisation published in English before October 18th 2013. We used the PRISMA statement for systematic reviews.[2] We performed an electronic PubMed, Cochrane and EMBASE database search. The terms plaster cast or hip cast brace or pantaloon cast or spica cast were used. Articles regarding children, dysplasia, congenital hip dislocation, Legg-Calvé-Perthes and immobilisation with plaster cast restricted to the lower limb were excluded. From the retrieved articles, the reference lists were screened for any relevant papers. Full-text copies of these articles were obtained and assessed for eligibility. Articles which did not describe the type of cast or brace and articles concerning treatment with traction or prolonged bed rest prior to bracing were excluded. All papers selected were analysed for incidence of DVT and pulmonary embolism.

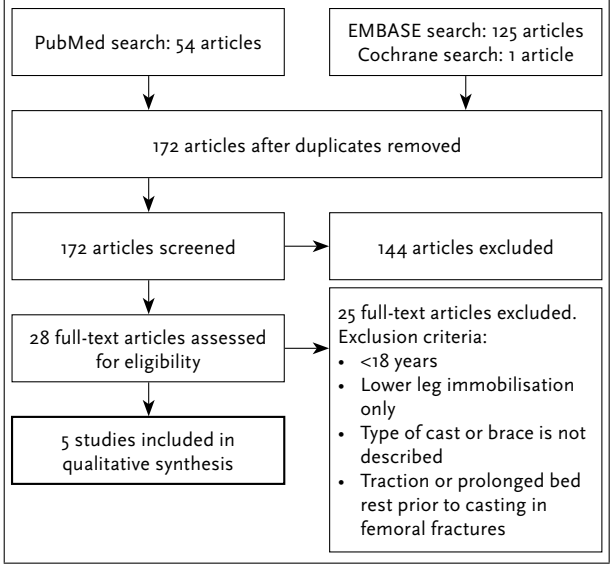
RESULTS

We reviewed 180 papers describing plaster cast immobilisation (*figure 1*). There were no randomised controlled trials or non-randomised comparative studies. Two review articles by the same author were identified.[3,4] One article described the author's thesis, including the other review article. The systematic review included three studies with a hip plaster cast test for surgical decision-making in 120 patients with chronic low back pain.[3] One of those three studies did not involve immobilisation of the hip. Rask *et al*. included 45 patients and the immobilisation period was four weeks initially and became two weeks later on.[5] Markwalder *et al*. immobilised 25 patients for two weeks.[6] In both studies, no VTE complications were described and the use of thromboprophylaxis was not reported.

Data of the review were supplemented by a prospective cohort study with 257 patients.[3] Casts were applied for 3-6 weeks. The use of thromboprophylaxis was not described and no VTE complications were reported. It was concluded that in patients without prior spine surgery a hip plaster cast test with substantial pain relief suggests a favourable outcome of lumbar fusion compared with conservative management.

The third article of our search described a cohort of 67 patients with a hip cast brace after primary and revision hip replacement in order to prevent hip dislocation.[7]

Figure 1. *Algorithm showing search method, according to PRISMA methodology*



Two patients developed deep venous thrombosis. This brace allowed 70 degrees of flexion and a variable range of abduction in the hip joint, however. The hip cast in this case report is designed to immobilise the hip joint in a fixed 10 degrees of flexion. The purpose of this cast is to simulate lumbar fusion. The same type of soft-cast brace was applied in the fourth article to 21 patients, to conservatively treat hip dislocation for three months after total hip arthroplasty.[8] No VTE was reported. The fifth article reported on 16 patients who were treated with a hip plaster cast for six weeks for dislocated total hip arthroplasty.[9] Three patients underwent additional revision hip arthroplasty before the cast was applied. No thromboembolic complications were described.

In summary, the overall incidence of symptomatic VTE during hip plaster cast immobilisation was 0%. The incidence of symptomatic VTE in hip cast brace was 2.3% (range 0-3%).

CASE REPORT

A 29-year-old woman with radiographically confirmed discopathy at level L4 to S1 was immobilised with a trial hip plaster cast immobilisation to simulate lumbosacral fusion. Pain relief would aid in the decision for lumbar fusion. The patient was on oral contraceptive medication (OCM) (ethinylestradiol 20 µg, desogestrel 150 µg). No other risk factors for the development of VTE were found. The patient's body mass index was 24. After 11 days of plaster cast immobilisation, the patient became dyspnoeic. Two days later, the cast was removed and the following day,

she presented to the emergency department with persistent dyspnoea and mid-sternal pain. There were no evident symptoms of deep venous thromboembolism of the legs. The diagnosis of pulmonary embolism was determined by means of a D-dimer of 15 mg/l in combination with multiple perfusion defects on a perfusion scan and confirmed by CT angiography, which showed a massive embolus in the right pulmonary artery and a central embolus in the left pulmonary artery. She was treated with low-molecular-weight heparin (LMWH) (nadroparin 5700 IE twice daily subcutaneously) and started with a vitamin K antagonist (VKA) (acenocoumarol). Once the international normalised ratio (INR) was between 2.5 and 3.5, the LMWH was discontinued and VKA was continued for three months. The patient was discharged home after 14 days in hospital. Two years later, a ventral spondylodesis at levels L4-S1 was performed.

DISCUSSION

Our systematic review of the literature did not show any symptomatic venous thromboembolic events during hip plaster cast immobilisation in 343 patients. Symptomatic DVT was reported in 2.3% (range 0-3%) of 88 patients immobilised with a hip brace.

The risk of VTE in patients with plaster cast immobilisation is not properly documented. A meta-analysis regarding six studies on lower leg plaster cast immobilisation showed a highly significant and clinically relevant reduction in asymptomatic events with LMWH prophylaxis compared with placebo or untreated controls (RR 0.58, CI 0.39-0.86, p=0.006).[1] The mean rate of VTE was reduced from 17.1 to 9.6% with the use of LMWH.

The authoritative American College of Chest Physicians (ACCP) guidelines give a grade 2C recommendation (based on low quality evidence) not to use pharmacological thromboprophylaxis in patients with isolated lower-leg injuries requiring leg immobilisation.[10] They suggest that results from higher-risk populations may, however, be reasonably extrapolated to patients at higher risk of DVT (who were excluded from the studies), particularly those with prior VTE.

Several grading systems to identify risk factors for VTE have been developed, which are the subject of debate. Limitations of these risk assessment models include lack of prospective validation, applicability only to high-risk subgroups, inadequate follow-up time, and excessive complexity, according to the ninth ACCP guidelines regarding non-surgical patients.[11-14] To our knowledge, no clinical trials evaluating VTE prophylaxis for medical outpatients have yet been published.[15]

Generally, immobilisation is considered a major risk factor for VTE. In our case the immobilisation induced by the hip plaster cast, combined with the use of OCM, puts our patient in the high-risk category.

Therefore we recommend that patients who are fitted with a hip plaster cast should be routinely screened for additional risk factors such as OCM use and a history of VTE. When risk factors are present, patients should be considered for pharmacological thromboprophylaxis.

Informed consent was obtained from the patient.

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ORIGINAL ARTICLE

Incidence of first acute myocardial infarction over time specific for age, sex, and country of birth

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ABSTRACT

Objectives: To study the age- and sex-specific incidence rates of first acute myocardial infarction (AMI) among first-generation ethnic minority groups (henceforth, migrant groups) and the Dutch majority population in the Netherlands during two time periods (2000-2004 and 2005-2010).

Methods: Through linkage of Dutch nationwide registers, first AMI events in the Dutch majority population and the major migrant groups living in the Netherlands were identified from 2000-2004 and 2005-2010. Absolute incidence rates were calculated within each age-sex-period-country of birth group.

Results: Regardless of ethnic background, AMI incidence rates were higher in men than in women and increased with age. Incidence significantly declined over time among the Dutch majority population (men: -26.8%, women: -26.7%), and among most migrant groups under study. It was only in Moroccan migrants that AMI incidence significantly increased over time (men: 25.2%, women: 41.7%). Trends differed between age categories, but did not show a consistent pattern. The higher AMI incidence in Surinamese men and women and Turkish and Indonesian men compared with the Dutch majority population persisted over time, but decreased with age and became absent after 70 years of age. Moroccans had a significantly lower incidence compared with the Dutch majority population during 2000-2004, which disappeared during 2005-2010.

Conclusion: Primary preventive strategies should focus on Surinamese men and women and Turkish and Indonesian men below 70 years of age. Future research is necessary to unravel the factors that provoke the increasing AMI incidence over time among Moroccans.

KEYWORDS

Acute myocardial infarction, age, ethnicity, sex, time trends

INTRODUCTION

Cardiovascular disease (CVD) is one of the main contributors to morbidity and mortality worldwide. In the Netherlands it is the number one cause of death in women and the number two in men, immediately after cancer. Ischaemic heart disease (IHD), and in particular acute myocardial infarction (AMI), is responsible for the majority of CVD deaths.[1] Information on age- and sex-specific incidence rates of AMI is vital in developing and maintaining preventive strategies. The latest detailed estimates of absolute AMI incidence in the Netherlands stratified by age and sex date back to 2000, and were restricted to the general Dutch population.[2] As a decline in AMI incidence has taken place in the Netherlands over the past decade, there is a need for updated AMI incidence estimates.[3]

Since 10% of the Dutch population was born abroad, and ethnic variations in AMI incidence have been reported, it is important to present results specific for country of birth.[4-7] Internationally, only a few studies have described absolute age- and sex-specific incidence rates in minority and migrant groups.[8-11] Results of these studies suggest that ethnic differences in AMI incidence are particularly present in the young, and that AMI incidence is higher in men than in women irrespective of age and country of birth. In the Netherlands, such estimates are unavailable yet. It is well known that AMI incidence has been declining over time in Western countries.[3,12] However, time trends among ethnic minority and migrant groups have not been

widely investigated, and age- and sex-specific time trends are even more limited.[11,13-16] The one study reporting such extensively stratified data showed a larger decline in AMI incidence among White Americans than among African Americans, especially in men and elderly women.[11] This information is important to monitor whether preventive strategies have gained effect, and in which groups this occurred. Furthermore, it may target specific groups that need extra attention in future preventive strategies. Therefore this study presents the absolute incidence rate of age- and sex-specific first AMI events (hospitalisations and out-of-hospital deaths) during two time periods (2000-2004 and 2005-2010) within the Dutch majority population and within migrant groups living in the Netherlands.

METHODS

Data sources and enrolment

New cases of first AMI events in the Dutch population were identified during two time periods: 2000-2004 (period one) and 2005-2010 (period two). This enabled us to investigate trend patterns in AMI incidence during the past decade, while keeping numbers high enough for analysis. First AMI events included hospitalised first AMI patients and out-of-hospital deaths from first AMI. Data on AMI hospitalisations were derived from the Dutch National Hospital Discharge Register (HDR). Data on out-of-hospital deaths from AMI were derived from the National Cause of Death Register (CDR). Demographic data (date of birth, country of birth, country of birth of parents, sex) were derived from the Dutch Population Register (PR), which contains information on all officially registered persons living in the Netherlands. The registers have been described in detail previously.[17] The overall quality of Dutch nationwide registers proved to be adequate.[18,19]

As the nominator, all persons with a first AMI event during period one and period two were included. A first AMI comprised all first hospitalisations with AMI as principal or secondary diagnosis (ICD-9 code 410) and all out-of-hospital deaths with AMI as primary or secondary cause (ICD-10 code I21). Persons who suffered a previous hospitalisation with AMI as the principal or secondary diagnosis from 1995 onwards were excluded. As denominator, person-years at risk were calculated based on all unique persons in the PR during the years 2000-2010 (if a person was unique in only a part of these years, person-years at risk were based on these years). A person was ascribed PR unique when there was a unique combination of the variables date of birth, sex, and four digits of postal code in the year of interest. In case of non-uniqueness a person could not be validly tracked down in the HDR and had to be excluded.

Ethnic background

Only first-generation ethnic minority groups were included (henceforth, migrant groups). Migrant groups were constructed based on the country of birth of the resident and his/her parents, according to the definition of Statistics Netherlands.[20] A person is considered a migrant if he/she was born abroad and at least one of the parents were born abroad. Persons in which both parents were born in the Netherlands were indicated as the Dutch majority population. For this study, individuals born in Suriname, Morocco, Turkey, Netherlands Antilles, and Indonesia were included (the five major migrant groups living in the Netherlands).

Data analysis

AMI incidence rates in period one and two with 95% confidence intervals (95% CI) were computed by age (30-39, 40-49, 50-59, 60-69, 70-79, 80-89 and ≥90) and sex within the Dutch majority population and within the migrant groups under study. The incidence rates were expressed as number of events per 100,000 person-years at risk. Subsequently, age-standardised incidence rates were calculated using the age distribution of the European population in ten-year age bands. The percent change in AMI incidence rate over the two time periods was calculated within each age-sex-country of birth group. To study the differences between migrant groups and the Dutch majority population, relative risks (RR) were calculated within each age-sex-period group (reference=Dutch majority population). We used SPSS software, version 14.0 (SPSS Inc, Chicago, Illinois, USA) to calculate the number of events and person-years at risk. Incidence rates with 95% CIs were calculated with the online program openepi.com.[21] Age-standardised incidence rates with 95% CIs were calculated with STATA 11.0 (Stata Corp. 2009. *STATA Statistical Software: Release 11*. College Station, TX: StataCorp LP). Relative risks and percent changes were calculated using Microsoft Excel 2010. All analyses were performed in accordance with privacy legislation of the Netherlands.

RESULTS

During 2000-2004, the mean number of first AMI events was 25,070 per year; from 2005-2010 this number decreased to 18,507 per year. Among migrants, the Antilleans were the smallest and the Surinamese the largest group *(results not shown)*. Men had the highest AMI incidence rate within all countries for birth groups, age strata, and time periods (*table 1 and 2*). Incidence increased with age in all groups and time periods under study.

Trends in AMI incidence among migrants and the Dutch majority population

There was a decline in AMI incidence over time among the Dutch majority population, among Surinamese and

Table 1. *Incidence rate of acute myocardial infarction per 100,000 person-years at risk in every age-sex group in the Dutch majority population and in the major migrant groups living in the Netherlands between 2000 and 2004*

	Majority population	Surinamese		Moroccan		Turkish		Antillean		Indonesian	
	IR	IR	RR	IR	RR	IR	RR	IR	RR	IR	RR
Men											
<30	1.4 (1.1-1.6)	-	-	-	-	-	-	-	-	-	-
30-39	31 (30-33)	81 (63-103)	2.6*	31 (21-46)	1.0	61 (48-76)	2.0*	29 (13-55)	0.9	-	-
40-49	141 (137-145)	318 (281-357)	2.3*	78 (51-113)	0.6*	283 (244-326)	2.0*	172 (127-228)	1.2	229 (187-278)	1.6*
50-59	334 (328-340)	573 (511-641)	1.7*	179 (126-247)	0.5*	571 (499-651)	1.7*	322 (243-417)	1.0	383 (342-429)	1.1*
60-69	581 (572-590)	698 (600-807)	1.2*	473 (366-602)	0.8	726 (641-819)	1.2*	624 (444-854)	1.1	642 (582-706)	1.1*
70-79	993 (979-1007)	782 (618-976)	0.8*	392 (223-643)	0.4*	681 (474-950)	0.7*	946 (526-1577)	1.0	996 (906-1093)	1.0
80-89	1500 (1472-1528)	1609 (1175-2155)	1.1	-	-	-	-	-	-	1372 (1201-1561)	0.9
≥90	1971 (1878-2067)	-	-	-	-	-	-	-	-	2424 (1748-3280)	1.2
All ages	248 (246-250)	293 (275-312)	1.2*	71 (61-82)	0.3*	220 (205-236)	0.9*	123 (106-143)	0.5*	574 (546-604)	2.3*
Age stand[a]	*205 (203-206)*	*270 (247-292)*	*1.3**	*111 (92-131)*	*0.5**	*228 (208-248)*	*1.1**	*196 (158-234)*	*1.0*	*235 (220-250)*	*1.1**
Women											
<30	0.5 (0.4-0.7)	-	-	-	-	-	-	-	-	-	-
30-39	10 (9-11)	16 (9.3-25)	1.6	-	-	12 (6.2-20)	1.2	-	-	-	-
40-49	43 (41-45)	53 (41-69)	1.2	-	-	48 (33-70)	1.1	-	-	44 (28-66)	1.0
50-59	81 (78-84)	154 (124-188)	1.9*	-	-	106 (78-142)	1.3	82 (48-130)	1.0	86 (68-109)	1.1
60-69	211 (206-217)	304 (247-372)	1.4*	225 (134-358)	1.1	213 (158-281)	1.0	302 (200-539)	1.4	236 (201-275)	1.1
70-79	500 (491-509)	590 (479-719)	1.2	-	-	420 (267-630)	0.8	304 (148-558)	0.6	523 (468-583)	1.0
80-89	986 (969-1002)	978 (744-1263)	1.0	-	-	785 (318-1633)	0.8	1059 (538-1888)	1.1	952 (850-1063)	1.0
≥90	1423 (1380-1467)	1060 (539-1890)	0.7	-	-	1579 (402-4297)	1.1	-	-	1335 (1076-1639)	0.9
All ages	147 (145-148)	106 (96-117)	0.7*	17 (12-23)	0.1*	48 (41-56)	0.3*	51 (40-63)	0.3*	297 (279-317)	2.0*
Age stand[a]	*86 (85-87)*	*108 (96-120)*	*1.3**	*48 (30-65)*	*0.6**	*86 (64-109)*	*1.0*	*71 (53-88)*	*0.8*	*88 (81-94)*	*1.0*

IR=Incidence rate per 100,000 person-years at risk; RR=relative risk of AMI incidence compared to the Dutch majority population; [a]Standardised to the age-distribution of the European population in ten year age-bands; *Significant difference compared with the Dutch majority population.

Indonesian men and women, and among Turkish and Antillean men (*figure 1*). Among Moroccans incidence increased over time, whereas it remained stable among Turkish and Antillean women. After age stratification, the direction of time trends was often similar between age categories, with some variation in magnitude (*figure 2*). An exception was in Moroccan men, where age-stratified results showed decreased, increased, as well as stable AMI incidence rates over time.

Difference in AMI incidence between migrants and the Dutch majority population

Surinamese men and women, as well as Turkish and Indonesian men, had a statistically significantly higher AMI incidence compared with their Dutch counterparts, which remained stable over time (*table 1 and 2*). After age stratification, the higher incidence decreased with age and only remained in those younger than 70 years of age. Moroccans had a statistically significantly lower AMI incidence compared with their Dutch counterparts during period one, whereas there was no difference observed during period two. Among Moroccan men this was mainly due to the fact that the lower incidence in 50-80 year olds disappeared over time. Among Antillean men and women, and Turkish and Indonesian women, there was no difference in incidence with the Dutch majority population. After age stratification, however, AMI incidence was higher in some age groups among Turkish and Indonesian women, but in period two only (*table 2*).

Table 2. *Incidence rate of acute myocardial infarction per 100,000 person-years at risk in every age-sex group in the Dutch majority population and in the major migrant groups living in the Netherlands between 2005 and 2010*

	Majority population	Surinamese		Moroccan		Turkish		Antillean		Indonesian	
	IR	IR	RR	IR	RR	IR	RR	IR	RR	IR	RR
Men											
<30	0.9 (0.8-1.1)	-	-	-	-	-	-	-	-	-	-
30-39	28 (27-30)	91 (72-114)	3.3*	16 (9.6-25)	0.6*	47 (37-60)	1.7*	39 (22-65)	1.4	-	-
40-49	116 (113-119)	238 (209-268)	2.1*	79 (60-102)	0.7*	220 (195-247)	1.9*	95 (66-132)	0.8	179 (128-243)	1.5*
50-59	266 (261-270)	458 (415-506)	1.7*	250 (194-317)	0.9	403 (350-462)	1.5*	261 (205-329)	1.0	361 (323-403)	1.4*
60-69	408 (401-414)	565 (497-640)	1.4*	443 (358-542)	1.1	501 (439-569)	1.2*	464 (357-593)	1.1	498 (450-551)	1.2*
70-79	665 (654-675)	571 (464-696)	0.9	760 (593-961)	1.1	524 (424-640)	0.8*	530 (314-843)	0.8	711 (643-784)	1.1
80-89	1106 (1086-1127)	1013 (742-1352)	0.9	906 (491-1540)	0.8	-	-	-	-	1180 (1051-1321)	1.1
≥90	1561 (1491-1632)	2227 (1171-3870)	1.4	-	-	-	-	-	-	1565 (1147-2088)	1.0
All ages	199 (197-200)	287 (270-304)	1.4*	101 (90-113)	0.5*	196 (183-209)	1.0	119 (103-136)	0.6*	517 (491-544)	2.6*
Age stand[a]	*150 (148-151)*	*211 (197-225)*	*1.4**	*139 (122-155)*	*0.9*	*170 (156-183)*	*1.1**	*132 (110-154)*	*0.9*	*180 (168-192)*	*1.2**
Women											
<30	0.6 (0.5-0.7)	-	-	-	-	-	-	-	-	-	-
30-39	9.1 (8.2-10)	-	-	-	-	10 (5.5-16)	1.1	-	-	-	-
40-49	37 (36-39)	47 (36-59)	1.3	19 (10-34)	0.5*	38 (28-51)	1.0	-	-	37 (20-62)	1.0
50-59	72 (69-75)	116 (97-139)	1.6*	46 (25-79)	0.6	112 (86-144)	1.6*	48 (28-78)	0.7	76 (60-95)	1.1
60-69	145 (141-149)	236 (196-282)	1.6*	221 (143-326)	1.5	216 (173-266)	1.5*	111 (67-174)	0.8	175 (147-207)	1.2*
70-79	350 (343-357)	398 (324-483)	1.1	423 (269-635)	1.2	275 (194-378)	0.8	260 (148-425)	0.7	400 (355-448)	1.1*
80-89	732 (720-745)	555 (417-723)	0.8*	-	-	691 (384-1152)	0.9	989 (563-1621)	1.4	732 (656-815)	1.0
≥90	1140 (1107-1173)	-	-	-	-	-	-	-	--	1002 (813-1223)	0.9
All ages	117 (116-118)	96 (88-105)	0.8*	26 (21-32)	0.2*	58 (51-66)	0.5*	41 (33-52)	0.4*	256 (240-273)	2.2*
Age stand[a]	*63 (63-64)*	*74 (67-81)*	*1.2**	*68 (49-87)*	*1.1*	*70 (59-81)*	*1.1*	*60 (44-75)*	*1.0*	*67 (61-72)*	*1.1*

IR=Incidence rate per 100,000 person-years at risk; RR=relative risk of AMI incidence compared to the Dutch majority population; [a]Standardised to the age-distribution of the European population in ten year age-bands; *Significant difference compared with the Dutch majority population.

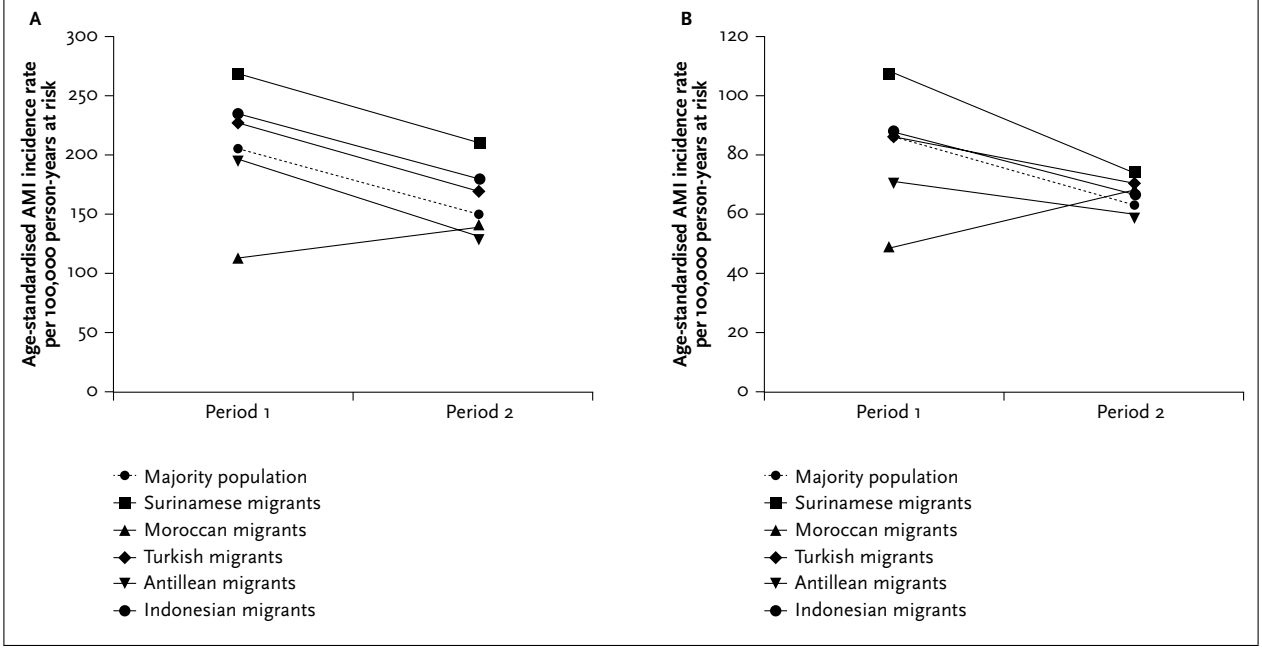
DISCUSSION

In every group under study AMI incidence increased with age and was higher in men than in women. All migrant groups, except Moroccans, showed a decreased or stable AMI incidence over time. The extent varied between age categories, depending on the country of birth. Among migrant groups with a higher incidence compared with the Dutch majority population, the difference diminished with increasing age, and disappeared in those older than 70 years of age. Among migrant groups with a similar or lower incidence compared with the Dutch majority population, there was no clear pattern over age categories. Among most age-sex-migrant groups, the difference with the Dutch majority population remained similar over time.

Key findings

There are some important findings that need to be addressed. Firstly, in migrant groups with a higher overall AMI incidence compared with the Dutch majority population (Surinamese men and women, Turkish and Indonesian men), the difference declined with increasing age. After the age of 70 years, a difference was no longer observed. This is in accordance with international literature studying aboriginal vs. non-aboriginal subjects in an Australian population, South Asian vs. White subjects in a Canadian population, and African-American vs. Caucasian American subjects in a US population.[8,10,22] Explanations for this phenomenon may include selective survival, which prevents those in the higher risk groups from reaching old age. Subsequently, elderly migrant

Figure 1. *Age-standardised AMI incidence rate in the Dutch majority population and migrants groups during period one and period two. A) Men; B) Women. Period one: 2000-2004, period two: 2005-2010*



groups are healthier compared with their younger counterparts, resulting in diminishing ethnic inequalities in AMI incidence among the elderly.[23] Literature also indicates that cardiovascular risk factors present themselves at an earlier age in migrants than in the majority population, which could lead to a shift in AMI incidence towards the young. For example, Surinamese women already had a hypertension prevalence of 35% at age 35, whereas their ethnic Dutch counterparts did not reach this level before the age of 45.[24] Similarly, another study showed that the prevalence of diabetes in the Dutch majority population started to increase from 55-64 years of age, while in Turkish and Moroccan migrants the same prevalence was already reached 10-20 years earlier.[25]

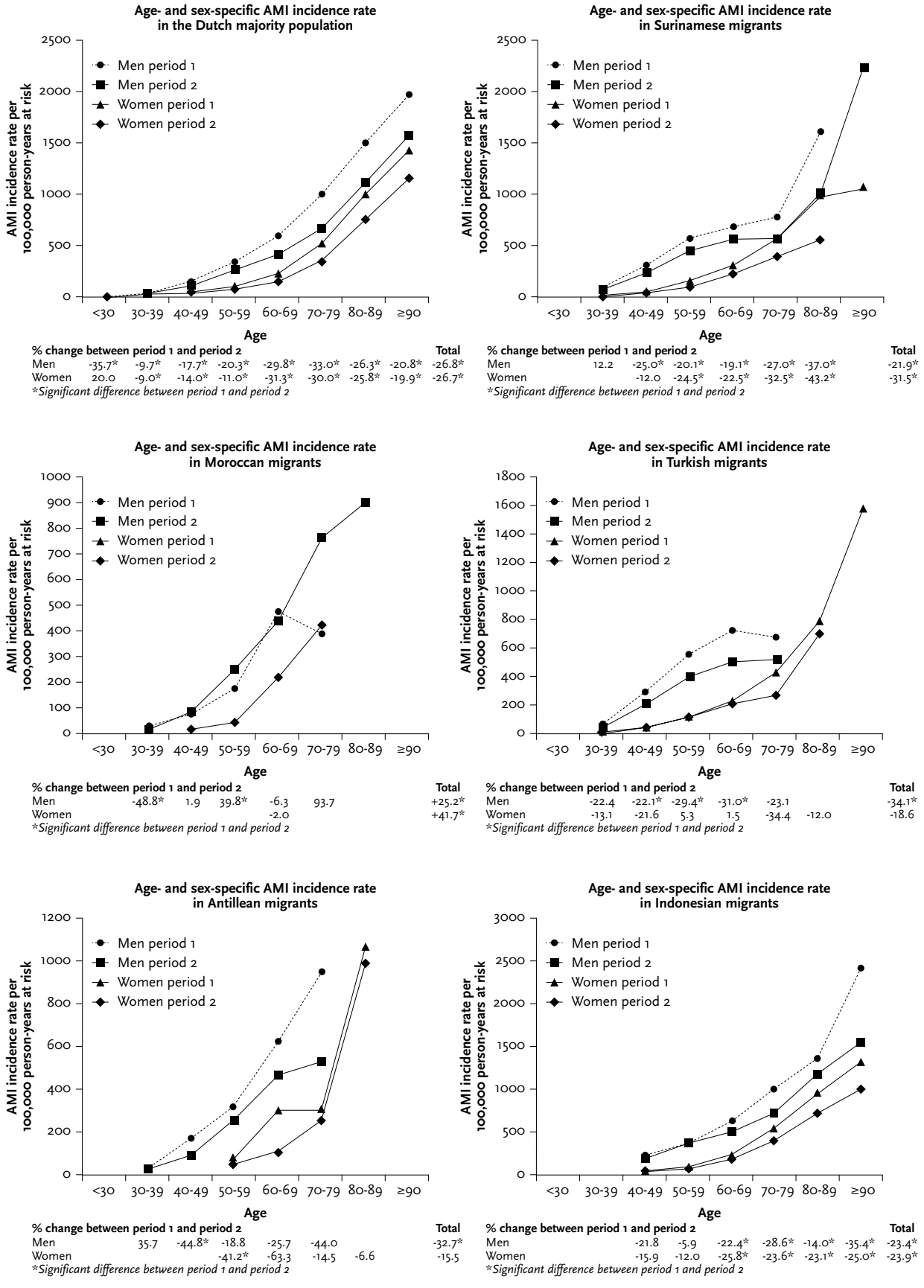
Secondly, Moroccans had a lower overall AMI incidence compared with the Dutch majority population in period one, whereas this difference disappeared in period two. In men this was caused by increasing incidence rates over time, especially in the 50-60 and the 70-80 year olds. In women, number of events was too small to distinguish time trends across age groups. One explanation for the increased AMI incidence among Moroccans is the very low initial AMI incidence rate, which may hinder the trend of further decline. Moreover, improvements in primary preventive efforts over the past years may have had less effect in Moroccans due to cultural and language barriers.[26-28] In addition, most improvements in the Netherlands were accomplished in the fields of cardiovascular drug use, smoking cessation and lowering total cholesterol, but not in reducing the major risk factors Moroccans have to deal with (obesity and diabetes).[29] Finally, previous literature indicates that migrants with an initially lower risk converge towards the risk of the majority population over time, provoked by the loss of healthy lifestyle factors and adoption of adverse lifestyle factors in the host country.[15,30] This was reflected in our study, since the AMI risk difference between Moroccans and the Dutch majority population narrowed from 0.5-0.9 in men, and from 0.6-1.1 in women between the two time periods (*figure 1*). The underlying factors of this adverse trend in AMI incidence among Moroccans need further attention in future research.

Thirdly, Turkish and Indonesian women had a similar overall age-standardised AMI incidence rate compared with the Dutch majority population. However, after age stratification, there was a significantly higher incidence observed in 50- to 70-year-old Turkish women and 60- to 80-year-old Indonesian women in period two. This was not observed in period one. The higher incidence in Turkish women was provoked by the absent decline in AMI incidence over time, especially in the 50- to 70-year-olds (*figure 2*). It is unclear why specifically these Turkish women show an adverse picture. Among Indonesian women between 60 and 80 years the significant difference with the Dutch majority population was only small and less relevant.

Considerations

Literature concerning absolute incidence rates of coronary heart disease by age, sex, and country of origin is scarce,

Figure 2. *Age- and sex-specific incidence rates during period one and period two by country of birth. Period one: 2000-2004; Period two: 2005-2010*



and in most cases reported results for one time period only.[8-10,22] Our study, stratifying by age, sex, country of origin, and time period, expands existing evidence. The nationwide registers yielded a large study population which made it possible to stratify by a wide range of determinants. The inclusion of primary as well as secondary diagnosis and causes of death decreased the chance of missed AMI events. The availability of hospital data from 1995 onwards provided a medical history varying from 5-9 years in the first period and from 10-15 years in the second period. This long medical history dramatically diminished the risk of misclassifying recurrent AMI events as first AMI events. Previous literature reported a five-year risk of recurrent AMI of 2.5% in both men and women, and a 12-year risk of 1.5% in men and 1.0% in women.[31] Because of the shorter medical history in period one compared with period two, incidence rates could have been slightly overestimated during period one. However, this overestimation will be minimal since the risk of a recurrent AMI only differed by 1% between a five- and a 12-year medical history.

Inevitably our study has some limitations. Firstly, incidence rates may have been underestimated because of misclassification of AMI events. However, validity of AMI registration in the HDR proved to be high with a positive predictive value of 97% and a sensitivity of 84%.[19] This means that 97% of all AMI cases in the HDR were correctly coded and that 84% of all AMI events in the Netherlands were registered in the HDR. Furthermore, the validity of AMI death in the cause of death register proved to be one of the highest of all causes, with a maximal misclassification of 10%.[18] By additionally including the secondary diagnosis and causes of death, misclassification was further limited. Secondly, persons who were not PR unique with respect to the combination of the variables date of birth, sex, and four digits of the postal code were excluded. The migrant groups under study have a higher risk on non-uniqueness, mainly due to the absence of the exact date of birth. Therefore, person-years at risk is underestimated, but since non-uniqueness was not related to AMI incidence it did not influence the absolute incidence rates.[32,33] Thirdly, a small number of the subjects could not be traced back completely between 1995-2000/2010 because they were not PR unique during the entire study period, or because they immigrated to the Netherlands during the study period. This could have led to a slight overestimation of first AMI events. Migrants are more likely to have migrated to the Netherlands during the study period, but since the majority of those who migrated after 1995 were too young to have suffered an AMI, differential overestimation of first AMI events is unlikely. Fourthly, from 2005 onwards, hospitals are no longer obliged to register in the HDR, which has led to about 10% missing AMI events between 2005 and 2010.[34] Subsequently, absolute incidence rates in the second period have been underestimated and declines over time may have been overestimated by maximally 10%. However, this percentage is not high enough to have influenced our final conclusions concerning trends in AMI incidence over time. The risks relative to the Dutch majority population also remain unaffected, since missing AMI events were evenly distributed within the entire Netherlands.

CONCLUSION

Regardless of ethnic background, AMI incidence increased with age and was higher among men than among women. Overall, all migrant groups, except Moroccans, showed a stable or declining AMI incidence over time. With respect to primary preventive strategies, health care professionals should focus on Surinamese men and women and Turkish and Indonesian men below 70 years of age, because of their high AMI incidence relative to the Dutch majority population. Future research should elucidate the factors that provoke the increasing AMI incidence over time among Moroccans.

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Netherlands
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CASE REPORT

Fatal microscopic pulmonary tumour embolisms in patients with breast cancer: Necessary knowledge for future medical practice

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ABSTRACT

Microscopic pulmonary tumour embolisms (MPTE) are a rare but life-threatening phenomenon in patients with a history of adenocarcinoma. Due to the nonspecific symptoms, diagnostic difficulty, and rapid progression, this condition is often fatal. We describe two patients who previously completed breast cancer treatment, and now present with fatal MPTE and we provide a comprehensive review of the literature.

KEYWORDS

Breast cancer, microscopic pulmonary tumour embolisms, metastatic disease

What was known on this topic?
Microscopic pulmonary tumour embolism (MPTE) is a highly underreported syndrome often occurring in patients with an adenocarcinoma. As the diagnosis is difficult, MPTE is frequently only reported at autopsy.

What does this add?
MPTE is treated by systemic agents directed against the primary tumour. If untreated, the outcome of MPTE is often fatal. Therefore, awareness of this syndrome amongst clinicians involved in the treatment of cancer patients is crucial in order to improve its outcome. The current manuscript provides, apart from case descriptions, an overview on the sparse literature available on this topic.

INTRODUCTION

Although pulmonary metastases are common in advanced breast cancer patients, microscopic pulmonary tumour embolisms (MPTE) are rarely diagnosed. When reaching the lungs, tumour cells escaped from the tumour vasculature can become trapped within pulmonary capillaries giving obstruction. In contrast to pulmonary thromboembolism, MPTE are not visible on a chest computed tomography angiography (CTA). Multiple occlusions of small vessels may produce pulmonary hypertension leading to right ventricular failure, ultimately resulting in death. Since the first description of MPTE in 1937,[1] it has been described in several different adenocarcinomas. MPTE is characterised by highly progressive pulmonary symptoms in patients with a history of malignant disease, but it can also be the first clinical manifestation of an occult carcinoma. Due to limitations of diagnostic tests and its rapid progression, MPTE is often only diagnosed by autopsy.

We report two breast cancer patients who died of right heart failure caused by MPTE and provide a comprehensive review of the literature on MPTE in breast cancer patients. A PubMed search resulted in the identification of 15 patients, published in English or Dutch over the past 50 years, including those presented below (*table 1*). They show a wide variability in time span between cancer diagnosis and development of MPTE symptoms. MPTE should be treated immediately with anticancer therapy, in contrast to the administration of anticoagulants in case of pulmonary thromboembolism.

Table 1. *Case reports published in English or Dutch in the last 50 years regarding breast cancer patients diagnosed with MPTE*

Reference number	Number of patients	Time since diagnosis primary cancer	First symptoms	Time to death since admission	Other metastatic sites
3	1	9 months	Dyspnoea on exertion, cough, flu-like syndrome, chest pain	Still alive after 17 months	None
4	2	2 years / 12 years	Dyspnoea since a couple of weeks / pain in the abdomen	3 weeks / 10 days	Skeletal and bone marrow Brain, ischaemia in ileum, kidney
5	1	2 weeks	Two weeks of dyspnoea	Several days	Lung, liver, bone marrow
6	1	3 years	Dyspnoea	3 days	Unknown
7	1	5 years	1 month of progressive cough and shortness of breath	7 days	Unknown
8	1	Diagnosis after death	4 weeks of progressive dyspnoea, fatigue, low-grade fever	5 days	Liver
9	1	1.5 years	5 days of dyspnoea and generalised weakness	Within one week	Bone, bone marrow and liver
10	3	Unknown	Unexplained dyspnoea	2-3 weeks of dyspnoea, died <1week of admission	All 3 patients had diffuse metastatic spread, including bone marrow
11	1	9 months	Dyspnoea	3 weeks after onset dyspnoea	Unknown
12	1	15 months	Dyspnoea, weakness, anorexia	6 months after onset dyspnoea	All other organs
Current manuscript	2	1 year / 12 years	Dyspnoea, muscle weakness, weight loss / dyspnoea and upper abdominal pain	12 days / 3 days	Bone and adrenal gland / liver

CASE DESCRIPTIONS

Case 1

A 69-year-old woman was admitted with rapidly progressive dyspnoea, weakness in her limbs and weight loss, 12 years after breast cancer diagnosis, pT1cN1M0, oestrogen receptor positive. Treatment consisted of an irradical lobectomy, followed by a mastectomy, adjuvant FEC chemotherapy (fluorouracil, epirubicin, cyclophosphamide), radiotherapy, and tamoxifen followed by letrozole. Since then, no evidence of recurrence was observed. We saw a pale woman experiencing shortness of breath, with a normal respiration rate, a saturation of 98% without oxygen suppletion, decreasing below 90% during exercise. No physical abnormalities were found. Routine laboratory analysis showed haemoglobin 6.4 mmol/l and thrombocytes 114 x 10^9/l (7.9 mmol/l and 182 x 10^9/l, respectively, two weeks earlier). Chest X-ray and CTA were performed without abnormalities and she had a normal electromyogram. Bone marrow aspiration showed infiltration of adenocarcinoma, with histopathological features similar to her previous breast cancer. A CT scan of chest and abdomen showed no localisation of metastatic disease. We immediately started chemotherapy; paclitaxel weekly intravenously 90 mg/m^2. Despite that, she developed progressive respiratory failure during the next day, and was admitted to the intensive care unit (ICU) where she developed progressive anaemia (haemoglobin 5.3 mmol/l), thrombocytopenia (platelets 24 x 10^9/l) and high lactate levels (13.7 mmol/l). An echocardiogram showed pulmonary hypertension with signs of right heart failure. Despite mechanical ventilation and haemodynamic support, she had a cardiac arrest and died two days after the first administration of chemotherapy. Autopsy showed extensive multiple pulmonary micrometastases with right ventricular failure as cause of death. Also multiple bone metastases and an adrenal metastasis were found.

Case 2

A 56-year-old woman was diagnosed with cT4N1M0 invasive ductal carcinoma of the breast, oestrogen receptor positive, progesterone receptor negative, and HER2 negative. Treatment consisted of neo-adjuvant chemotherapy (six cycles of docetaxel, doxorubicin and cyclophosphamide), resulting in a clinical complete response, followed by a mastectomy yielding a 0.4 cm ductal carcinoma), axillary lymph node dissection in which no tumour localisation was detected (ypT1aN0), local radiotherapy and adjuvant tamoxifen. One year after diagnosis she was admitted with rapidly deteriorating

dyspnoea and abdominal pain in the right upper quadrant for one week. Liver enzymes were mildly elevated, lactate dehydrogenase was highly abnormal (2233 U/l; ULN 250 U/l), and cancer antigen 15-3 was elevated (98 E/ml). Three days after admission she developed respiratory failure and was admitted to the ICU. CTA imaging showed no pulmonary embolisms or other causes of pulmonary hypertension. An echocardiogram showed severe right heart failure. There was pulmonary hypertension, measured using a Swann-Ganz catheter. Blood tests showed a metabolic acidosis with high serum lactate (13.9 mmol/l), thrombocytopenia (platelets 42 x 10^9/l) and anaemia (haemoglobin 7.4 mmol/l). The condition of the patient did not warrant cytotoxic treatment. Despite maximal treatment she died of respiratory and haemodynamic failure. Autopsy revealed multiple microscopically small intravascular carcinomatous embolisms in the small arterioles of the pulmonary vasculature and in the sinusoids of the liver. In the right ventricle no signs of chronic failure or cardiomyopathy were detected, corresponding to the sudden onset. The presumed cause of death was right ventricular failure due to extensive pulmonary tumour emboli. The high serum lactate was probably due to circulatory failure resulting in anaerobic metabolism in the peripheral tissue. The low platelet count may be explained by the massive emboli resulting in high platelet use or by diffuse intravascular coagulation but other parameters necessary for this diagnosis, such as fibrogen and fragmentocytes, were not tested.

DISCUSSION

One of the first signs of MPTE is unexplained dyspnoea, with a rapidly progressive course within days or weeks. Differential diagnostics include lymphangitic carcinomatosis, vascular compression by a tumour, pulmonary thromboembolisms, pneumonia, pleural effusion, pericardial effusion, and heart failure e.g. chemotherapy-induced cardiotoxicity. Although only occasionally reported in literature, MPTE is thought to be underreported. An autopsy study of breast cancer patients reported that pulmonary tumour embolisation was the primary cause of death in four out of 100 patients reviewed.[2] Reasons for missing the diagnosis of MPTE are lack of a sensitive test, as it cannot be found with CTA imaging or with laboratory analysis. MPTE should be considered after excluding the more common diagnoses, of which the most important is pulmonary embolism. The definite diagnosis can only be made by tissue examination. In patients with symptoms suggestive of MPTE in which no other diagnosis is made and with a (history of) malignancy, obtaining tissue, for example by means of transbronchial fine needle aspiration should be considered for the definite diagnosis. If untreated, MPTE may lead to the occlusion of peripheral pulmonary vasculature causing pulmonary hypertension with subsequent right ventricular failure and ultimately cardiovascular collapse. Although often fatal, survival of a breast cancer patient with MPTE treated with effective chemotherapeutics has been reported,[3] proving that adequate treatment can be lifesaving. The treatment of choice is a cytotoxic regimen directed against the primary tumour, in this case breast cancer. MPTE can present as a manifestation of massive metastatic disease, but also as the only site involved. The aggressive nature does not leave much time for diagnostics, but if until recently the patient had a good performance status, limited disease localisation, and relevant systemic treatment options are available, the urgent initiation of systemic treatment should certainly be considered. *Table 1* summarises the currently available descriptions of patients with MPTE. Although it may be the first presentation of cancer, most patients have been treated for breast cancer years before developing MPTE. As discrimination between MPTE and pulmonary thromboembolisms is difficult, the diagnosis often remains unclear and instead of treating the underlying malignancy, anticoagulation for suspected thromboembolism is given, which is ineffective. Autopsy, if performed, then reveals the MPTE.

CONCLUSION

MPTE is a rare insidious syndrome presenting with progressive dyspnoea and pulmonary hypertension in patients with widespread incurable cancer, a history of malignant disease or occasionally as the first symptom of occult malignancy, often breast cancer. Despite papers reporting high prevalence in autopsy series, MPTE is still highly unrecognised due to its difficulty to diagnose. The clinical diagnosis can be made in patients with dyspnoea for which no explanation is found on CTA and after cardiac evaluation and with other signs or symptoms of metastatic disease. Awareness of possible MPTE is of high importance as, if clinically possible, it is essential to initiate immediate cytotoxic treatment as this is the only possible treatment of this often fatal syndrome.

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PHOTO QUIZ

Spots inside out

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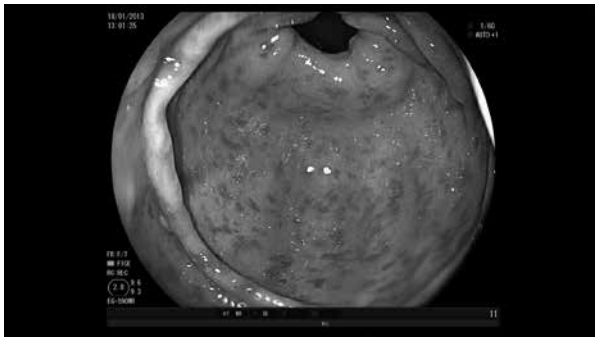
CASE REPORT

A 73-year-old male patient presented with dyspnoea, coughing, loss of appetite because of abdominal discomfort, bloody stools and a spontaneous haematoma on the buttocks. In the weeks prior to admission the patient had been treated with two different antibiotics and glimepiride had recently been started for diabetes mellitus. His further medical history included tuberculosis, alcohol abuse, morbid obesity and COPD. Physical examination revealed petechiae on his ankles and lower back and a sizeable haematoma on the buttocks. Laboratory results showed a slightly elevated C-reactive protein (76; n ≤6 mg/), leucocytosis (20.6; n=4-11 x 10^9/l) normal platelet count (313; n=150-400 x 10^9/l) and acute on chronic kidney failure (creatinine=168; 95 previously; n=50-110 µmol/l), no proteinuria (urine protein/creatinine ratio 0.12; n<0.13) and microscopic erythrocyturia. Because of the abdominal pain and melaena, a duodenoscopy was performed which showed purpuric lesions of the antrum (*figure 1*)

Figure 1. *Purpuric lesions of the antrum*



WHAT IS YOUR DIAGNOSIS?

See page 36 for the answer to this photo quiz.

Netherlands
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A brown-eyed woman with blue discoloration of the sclera

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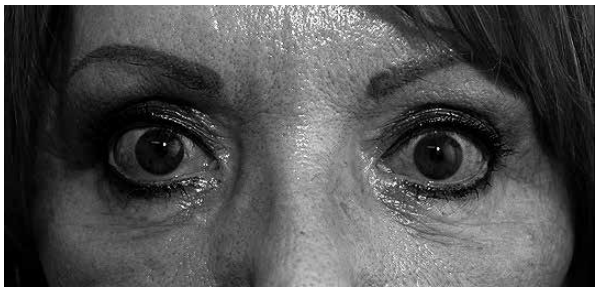
CASE REPORT

A 59-year-old woman presented to the outpatient department with blue discoloration of the sclera which had been slowly progressive over the last three years. She also reported diffuse hyperpigmentation of the skin. There were no associated symptoms. She was on thyroid hormone replacement because of hypothyroidism diagnosed three years earlier. Furthermore, she had been taking oral minocycline daily as therapy for acne vulgaris for more than 20 years.

Local examination revealed bilateral blue pigmentation of the sclera (*figure 1*). The visual function was intact. Furthermore, diffuse brownish pigmentation of the skin was noticed. The routine blood investigations, including serum ferritin and TSH levels, were within normal limits.

Figure 1. *Local examination revealed bilateral blue pigmentation of the sclera*



WHAT IS YOUR DIAGNOSIS?

See page 37 for the answer to this photo quiz.

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Oesophageal dilatation with pulmonary consolidation

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CASE REPORT

A 78-year-old woman presented with cough and expectoration. She also complained of dysphagia and regurgitation for the last three months. She had used mineral oil for a long time to treat constipation. Physical examination revealed bilateral crackles and an SaO_2 of 95%. Computed tomography (CT) demonstrated bilateral consolidation with areas of low attenuation and the presence of oesophageal dilatation (*figure 1*). Endoscopy showed oesophageal achalasia. Laboratory tests yielded serological findings of Chagas disease (CD), and indirect immunofluorescence and an enzyme-linked immunosorbent assay were positive. Bronchoalveolar lavage demonstrated the presence of intrapulmonary lipids (*figure 2*). The patient was treated with nifurtimox and physiotherapy, and was discharged for outpatient monitoring.

Figure 1. *A) HRCT at the level of the upper lobes with lung window settings demonstrated bilateral areas of consolidation surrounded by ground-glass attenuation, and thickening of the interlobular septa. B) CT image with mediastinal window settings showing areas of low attenuation within consolidations, better identified on the left (white arrows). Note also the presence of oesophageal dilatation (arrowheads)*

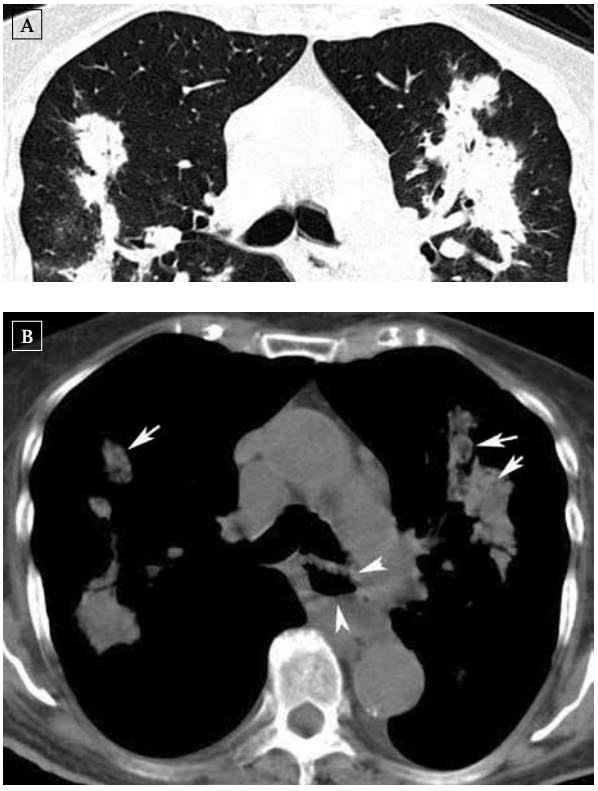
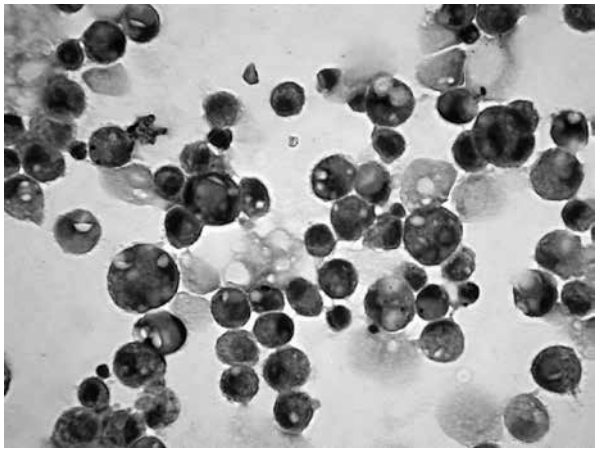


Figure 2. *Alveolar macrophages recovered by bronchoalveolar lavage and stained with oil red O. The cytoplasm is full of large rounded vacuoles that displace the nucleus to the periphery (oil red O stain, ×400)*



WHAT IS YOUR DIAGNOSIS?

See page 38 for the answer to this photo quiz.

Netherlands
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PHOTO QUIZ

A man with 'black fingers'

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In December, a 76-year-old male presented himself to the emergency room with 'black, painful fingers', which had developed within days. The patient had no prior medical history. He had no specific complaints, was not taking any medicine and had no intoxications. Physical examination of the hands showed a black discoloration on digits II-V of both hands with a relatively sharp demarcation at the 2nd phalanges. Laboratory tests of the blood were hampered due to direct 'clotting' of the blood in the test tubes.

Figure 1. *Hands showing black discoloration on digits II-V*

WHAT IS YOUR DIAGNOSIS?

See page 39 for the answer to this photo quiz.

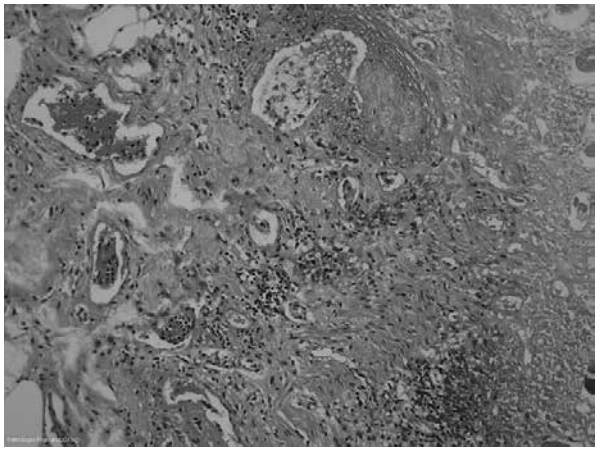
ANSWER TO PHOTO QUIZ (PAGE 32)

SPOTS INSIDE OUT

DIAGNOSIS

Biopsies from the antrum and duodenum showed mild inflammation. The definitive diagnosis was made after a biopsy of the haematoma on the buttocks, which showed a perivascular infiltrate of mixed cells (leukocytoclastic vasculitis) with vascular deposits positive for IgA on immunofluorescence consistent with IgA vasculitis (*figure 2*). IgA vasculitis/Henoch-Schönlein purpura (IgAV) is a small-vessel vasculitis with IgA immune complex depositions. It is characterised by a tetrad of clinical manifestations: palpable purpura (lower limb predominance), arthritis/arthralgia, diffuse abdominal pain and proteinuria/haematuria.[1] It is a typical childhood disease with an incidence of 17:100,000 but it may be seen in adults. It generally occurs outside the summer season and half of the episodes are preceded by an upper respiratory tract infection.[2] A variety of infectious, genetic and chemical triggers are suggested as causes of IgAV.[3]
Because of deteriorating renal function, the patient was treated with methylprednisolone. On the 12th hospital day he was transferred to the intensive care unit (ICU) because of an unexplained coma later shown to be caused by metabolic encephalopathy (serum ammonia 246; n <30 µmol/l). On the ICU he died within 24 hours due to multiple organ dysfunction. At autopsy complete ischaemia of the jejunum and ileum was found probably due to an arterial occlusion caused by extensive atherosclerosis of the abdominal circulation, although a microvascular cause (IgAV) could not be ruled out. A similar case report was recently published.[4] Unfortunately, immunofluorescence or microscopy of the kidney was not possible because of autolysis.

Figure 2. *Perivascular infiltration*



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ANSWER TO PHOTO QUIZ (PAGE 33)

A BROWN-EYED WOMAN WITH BLUE DISCOLORATION OF THE SCLERA

DIAGNOSIS

Minocycline is a yellow coloured, semi-synthetic tetracycline antibiotic that turns black when oxidised. It can induce severely disfiguring discoloration of the skin, nails, oral mucosa, ear cartilage, conjunctiva, teeth, bones, thyroid gland and pigmentation of heart valves.[1,2] These side effects are associated with long-term use of this drug, for example as treatment for acne vulgaris, rosacea or rheumatoid arthritis. Minocycline-induced hyperpigmentation is thought to occur in 14.8% of patients with acne vulgaris or rosacea (median duration of treatment of 17 months) and in 41% of patients with rheumatoid arthritis after a median of 12 months.[3,4] Different patterns of hyperpigmentation of the skin can be distinguished based on histopathology: type I is characterised by blue-black discoloration in areas of previous inflammation or scarring; type II appears as blue-grey pigmentation of previously normal skin of the arms and legs; type III consists of symmetrical muddy-brown macules that is most prominent on sun-exposed areas.[1] The type of scleral deposits is not known because scleral biopsy to examine these deposits has never been performed.

The discoloration of the sclera typically is blue, but it has also been described as dark metallic blue, brownish or black.[5] It may be enhanced in light-exposed areas. Scleral hyperpigmentation is not usually an isolated manifestation and is frequently seen in combination with pigment changes as noted above. It usually resolves with discontinuation of minocycline, although resolution may take months to years, or it may be permanent.

In our case, minocycline was discontinued to prevent further pigmentation. No alternative treatment for acne was started and subsequently, five years later, there was still no improvement in the discoloration of the eyes.

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ANSWER TO PHOTO QUIZ (PAGE 34)

OESOPHAGEAL DILATATION WITH PULMONARY CONSOLIDATION

DIAGNOSIS

These tomographic and cytological findings were consistent with the diagnosis of exogenous lipoid pneumonia (ELP) in a patient with Chagas disease (CD). ELP is a rare disorder caused by the aspiration of mineral, vegetable, or animal oil. The most common cause of ELP is the use of mineral oil to treat constipation.[1,2] Diagnosis is based on a history of mineral oil ingestion; consistent radiological findings, especially the finding of foci of fat attenuation within areas of consolidation on CT; and the demonstration of lipid-laden macrophages in bronchoalveolar lavage fluid or lung biopsy specimens.[1,2] CD is a common South American disease caused by the protozoan *Trypanosoma cruzi*, which predominantly results in alterations in the oesophagus, colon, and heart.[3,4] Chronic constipation is a common symptom in patients with CD and is often treated with mineral oil. The aspiration of mineral oil is not uncommon in these patients because of the presence of Chagas disease.[1,2] The association of these two factors (use of mineral oil for the treatment of constipation, and a predisposition to aspiration due to megaoesophagus) potentiates the development of lipoid pneumonia in patients with CD.

The diagnosis of ELP should be considered in chagasic patients with megaoesophagus who have used mineral oil to treat constipation and who present with parenchymal consolidations on CT.

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ANSWER TO PHOTO QUIZ (PAGE 35)

A MAN WITH 'BLACK FINGERS'

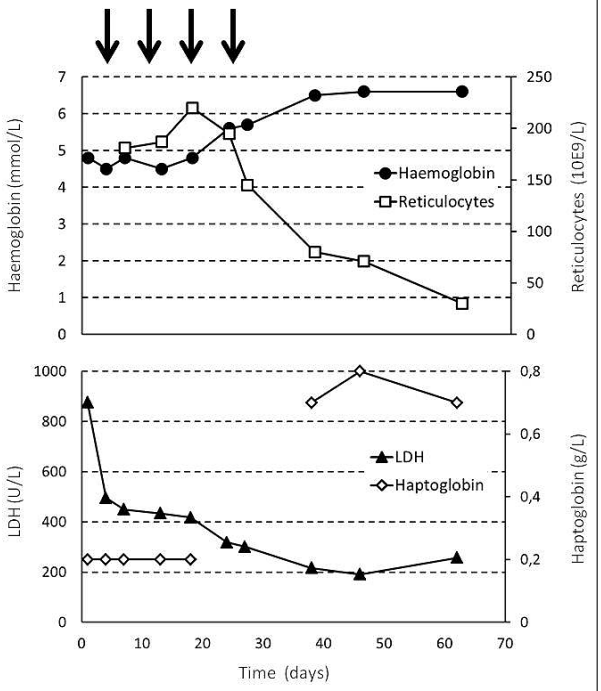
DIAGNOSIS

The apparent 'clotting' of the blood immediately after collection at room temperature was strongly suggestive of either the presence of cryoglobulins or cold agglutinins. Indeed, by pre-analytic handling and performing the laboratory tests at 37° C, agglutination could be avoided. These laboratory tests revealed the following: haemoglobin 4.8 mmol/l; leucocytes 14.4 10^9/l; reticulocytes 181.4 10^9/l; lactate dehydrogenase 877 U/l; haptoglobulin <0.2 g/l; and direct bilirubin 38 µmol/l. The direct antiglobulin test was strongly positive for C3d and negative for immunoglobulins (anti-IgG/IgM/IgA). An autoantibody with an anti-I specificity was found. No cryoglobulins were detected. Based on the clinical picture characterised by acrocyanosis and autoimmune haemolytic anaemia (AIHA) in the presence of cold antibodies, the diagnosis of cold agglutinin disease (CAD) was made.

CAD is a rare disease, which is either idiopathic (often precluding an occult lymphoma) or secondary to a haematological malignancy or infection (mycoplasma/EBV).[1] Cold antibodies are IgM auto-antibodies which bind to red blood cells at temperatures below 37° C. These antibodies are frequently directed against the antigen 'I', a carbohydrate structure present on red blood cells. Incidental cold agglutinins (polyclonal) can be found at a fairly high incidence, often with a benign nature. In CAD the optimal binding temperature of IgM auto-antibodies covers a broad range (broad thermal amplitude). In patients suffering from CAD, exposition of extremities to temperatures around the optimal binding temperature of the autoantibodies (e.g. around 30° C) may result in red blood cell agglutination in the microcirculation, a phenomenon which becomes clinically apparent by Raynaud's phenomenon and acrocyanosis. The closer the thermal amplitude shifts towards 37° C, the higher is the chance and efficacy of complement activation and hence the complement-mediated haemolysis.[2]

The basic rule in treatment of CAD is 'keep-it-warm'. Usually, patients with CAD suffer from a mild anaemia and there is no need for correction. However, when anaemia is more severe, treatment depends on the underlying cause. In case of an infection, CAD is often self-limiting. In CAD secondary to a haematological disease, this disease should be treated. For an idiopathic CAD, optimal treatment strategies have not been defined. Steroids and splenectomy – fairly effective in AIHA caused by warm auto-antibodies – have shown little effect in CAD.[3] Several small studies have shown a beneficial effect of rituximab in about 50% of the patients with CAD; although relapses frequently occur (usually within a year).[4,5] Addition of fludarabine seems to improve the response rate significantly, but toxicity can by considerable.[6]

Figure 2. *Haematological response after the administration of rituximab (depicted by arrows)*



In this patient a small (3%) monoclonal B cell population (IgM kappa) was detected. Given the serious acrocyanosis and ongoing haemolysis (*figure 2*) in the absence of an overt lymphoma, we chose to treat this patient with four doses of rituximab. After a month, the haemolysis halted (*figure 2*) and the haemoglobin level increased. The IgM kappa M protein was undetectable. In addition, the acrocyanosis did not expand further. Unfortunately, however, the pre-existing acrocyanosis was so severe that the upper phalanges could not be rescued and needed to be amputated.

This case demonstrates that haemolysis and acrocyanosis caused by cold antibodies in a patient with an underlying monoclonal B cell population can be halted by rituximab. Moreover, this case illustrates that the proper diagnosis in

CAD requests appropriate pre-analytic handling of patient samples.

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SPECIAL ARTICLE

Delivering high-quality care to patients with a non-Hodgkin's lymphoma: barriers perceived by patients and physicians

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ABSTRACT

Background: Despite the presence of non-Hodgkin's lymphoma (NHL) guidelines, there are still gaps between best evidence as described in guidelines and quality of care in daily practice. Little is known about factors that affect this discrepancy. We aim to identify barriers that influence the delivery of care and to explore differences between patients' and physicians' experiences, as well as between the different disciplines involved.

Methods: Patients and physicians involved in NHL care were interviewed about their experiences with NHL care. The barriers identified in these interviews were quantified in a web-based survey. Differences were tested using Chi-square tests.

Results: Barriers frequently perceived by patients concerned lack of patient information and emphatic contact (12-43%), long waiting times (19-35%) and lack of guidance and support (39%). Most barriers mentioned by physicians concerned the unavailability of the guideline (32%), lack of an up-to-date guideline (66%), lack of standardised forms for diagnostics (56-70%) and of multidisciplinary meetings (56%). Perceived barriers concerning the guideline and standardised forms significantly varied between the disciplines involved (range 14-84%, $p<0.05$).

Conclusion: Patients and physicians experienced different barriers for high-quality NHL care. A tailored strategy to optimise guideline adherence and daily NHL care, based on these barriers, has to be developed and tested.

KEYWORDS

Barriers, implementation, non-Hodgkin's lymphoma, qualitative analyses, quality of care, quantitative analyses

INTRODUCTION

The incidence of malignant lymphoma has increased significantly over the past years.[1,2] Malignant lymphomas can be classified into Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). The latter is the most common haematological neoplasm in adults worldwide.[3] Multidisciplinary evidence-based guidelines for NHL have been developed, both nationally and internationally, to assist physicians and patients in their decisions regarding appropriate diagnostics, treatment and follow-up.[4-7]

Unfortunately, just the publication and dissemination of guidelines is often not enough to close the existing gap between guidelines and daily practice.[8] We believe that better guideline adherence can lead to a higher quality of care. Therefore, the first step in improving quality of care is getting insight into current daily practice and the factors that influence the delivery of high quality of care.[9]

For NHL, several studies have demonstrated that the patient care is suboptimal.[10-12] Wennekes *et al.*,[12] for example, described lack of guideline adherence concerning diagnostics, therapy and follow-up. However, less is known about barriers that influence delivery of high-quality NHL care experienced by patients and physicians. The aim of

this study is to identify the most important barriers that influence daily NHL care as perceived by patients and physicians.[13]

METHODS

Study design

We qualitatively explored barriers of delivering NHL care by performing semi-structured interviews among patients and physicians. In order to assess the importance of the barriers found, we quantified the barriers in a web-based survey.

Participants and recruitment

Patients. Patients were recruited for the interviews through the website of the Dutch Lymphoma Organisation (patient association (LVN)), or by their attending physician. For the surveys, Twitter and the online forum of the LVN were used for recruitment. Patients diagnosed before 2008 were excluded to ensure information on current quality of care (2008-2011). Patients were reminded to complete the survey by an updated news item on the LVN website and by another tweet. Consent for the interview and survey was presumed if patients responded positively.

Physicians. Physicians involved in NHL care, including haemato-oncologists, pathologists, radiation oncologists, radiologists and nuclear medicine physicians, were included in the study. For the interviews, physicians from 22 hospitals involved in an NHL study in 2006[12] were invited to participate. Additionally, physicians involved in the Lymphoma Working Party of the Haemato-Oncology Foundation for adults in the Netherlands (HOVON) were invited by e-mail. For the surveys, the Dutch Societies of Internal Medicine (NIV), Pathology (NVvP), Radiology (NVvR), Nuclear Medicine (NVNG), and Radiation Oncology (NVRO) were consulted for contact information. Based on this, physicians were contacted either via a call in the newsletter (NVvP, NVvR), by email (NVNG, NVRO), or by post (NIV). Since no additional registration exists for physicians specialised in NHL, all members of the Dutch Societies were contacted. A reminder was sent to all, two to four weeks after the initial mailing. The surveys were independently tested by two project members before fielding the questionnaires. Consent for the surveys was presumed if the questionnaire was completed.

Instrument development and content

Interviews. The interviews were scheduled according to the participants' preferences concerning date and setting (face-to-face or by telephone). Participants were asked about their experiences with clinical practice regarding NHL care. The structure of the interviews was based on previously developed quality indicators[12] and two theoretical models.[14,15] These models facilitate description of potential barriers using five domains: factors related to the guideline (I), to physicians (II) and patients (III) and factors concerning the organisational (IV) and social (V) context. Data collection was finished when no new influencing factors were found and saturation was reached. All interviews were audiotaped and transcribed verbatim for analysis with Atlas.ti® (version 6.2.23, Atlas.ti Scientific Software Development GmbH; Berlin, Germany). The results of the interviews were used for the surveys.

Surveys. Because patients and physicians have different perspectives in NHL care, two different surveys were developed. The surveys were converted into a web-based survey using LimeSurvey (version 1.91, Boston, MA). The online survey did not accept unanswered questions and adaptive questioning was used. A modified version of the 'Consumer Quality Index (CQI) for cancer patients' was used.[16] The CQI, based on the American Consumer Assessment of Health Providers and Systems (CAHPS) instrument, is a standardised method to measure experiences of patients concerning quality of care. Permission to use this survey was obtained.

The patients were asked about their experiences regarding the organisation of NHL care, competence of physicians, information provision and communication, collaboration in NHL care, guidance and support, and after care. Questions were scored using closed questions with four answer possibilities, including never (1), sometimes (2), most of the time (3) and always (4). When relevant, 'I don't know' or 'not applicable' were included. The first part of the survey contained 11 questions about characteristics of the patients, including age, gender and type of NHL.

The surveys developed for physicians consisted of 85 questions for haemato-oncologists, 52 for pathologists, 63 for radiologists and nuclear medicine physicians, and 59 for radiation oncologists. The first part of the survey contained eight questions about characteristics of the physicians and their clinical setting, including age, gender and the type of hospital. The surveys were divided into the same five domains as the interviews,[14,15] and concerned statements about the Dutch NHL guideline and local protocols, working according to the recommendations, the organisation of NHL care and the social context. The statements were scored on a five-point Likert scale (1=strongly agree, 5=strongly disagree).

Data analysis

Interviews. The interview transcripts were analysed using qualitative content analyses, taking into account the direct as well as the underlying meaning of the text.[17] Potential

barriers were identified independently by two members of the project team. Any discrepancies were discussed until consensus was attained. Two other members of the project team randomly choose two transcripts to verify the qualitative analyses. After extraction, the established barriers were categorised into the above-mentioned domains and their frequency was scored.

Surveys. SPSS (version 16.0, Chicago, IL) was used for the analyses of the survey results. The answer possibilities of the patient survey were dichotomised as disagreement (score 1 or 2) and agreement (score 3 or 4). For physicians, the Likert scores were classified as agreement (score 1 or 2), neutral (score 3) and disagreement (4 and 5). Differences between patients and physicians were descriptively reported. Chi-square tests (statistical significance set at two-sided $p<0.05$) were performed to get insight into differences in perceived barriers between the four disciplines involved in NHL care.

RESULTS

Participants and recruitment

Seventeen patients and 33 physicians from hospitals spread over the Netherlands were interviewed. *Table 1* shows the characteristics of 28 patients and 132 physicians who filled in the survey. Patients and physicians from all age groups were represented and the two sexes were equally divided in both patients and physicians. Most patients (19 out of 28) in our study population had a diffuse large B-cell lymphoma or follicular lymphoma.

Barriers perceived by patients and physicians

The interviews resulted in barriers in all five predefined domains. In total, during the interviews 62 unique barriers were identified by patients (24 barriers) and physicians (47 barriers). They mainly indicated barriers in the physician (24 barriers) and organisational domain (15 barriers). Physicians also mentioned 13 barriers concerning the guideline. Eight unique barriers in the social context and two barriers related to the patient were identified.

In *table 2a and 2b*, the most important barriers perceived according to patients and physicians, as quantified by the surveys, are summarised. Barriers are included if at least 15% of the responders classified that item as a barrier and if ≥15 responders answered the question concerned. Barriers among patients, physicians, and differences between them are described below. Some illustrative quotations from the interviews are included in *figure 1*.

Table 1. *Characteristics of patients and physicians who filled out the survey*

Characteristics	Patients (n=28)		Characteristics	Physicians (n=132)	
	N	%		N	%
Gender			**Gender**		
Male	14	50	Male	72	55
Female	14	50	Female	60	45
Age groups			**Age groups**		
25-44	8	29	25-44	62	47
45-64	18	64	45-64	67	52
≥65	2	7	≥65	2	2
NHL classification			**Discipline**		
Follicular lymphoma	9	32	Haematology/Oncology	50	38
DLBCL	10	36	Pathology	31	24
Marginal zone B-cell lymphoma	2	7	Radiation oncology	36	27
Other classification	7	25	Radiology/Nuclear medicine	15	11
Region of hospital			**Region of hospital[a]**		
North	3	12	North	14	11
East	2	8	East	25	19
South	3	12	South	7	5
West	18	69	West	82	62
			Abroad	4	3
Type of hospital[b]			**Type of hospital**		
University	10	38	University	45	34
Non-university	21	81	Non-university	87	66
Level of education			**Years of experience**		
Low	13	48	0-5 year	49	37
Middle	10	37	6-15 year	49	37
High	4	15	16-25 year	26	20
			>25 year	8	6

DLBCL = diffuse large B-cell lymphoma; [a] physicians were asked in which region they were trained; [b] five patients were under treatment in more than one hospital.

(I) Guideline-related barriers. No barriers related to the guideline (developed for and by physicians) were mentioned by the patients. Physicians pointed out barriers mainly regarding the lack of availability of local NHL protocols (47%) and lack of an up-to-date version of the NHL guideline (66%). They agreed about the need to have

Table 2a. *Most important perceived barriers that influence daily NHL care according to patients*

Perceived barriers per domain	Number of patients[a]	
(I) Guideline	**N**	**%**
-		
(II) Physicians	**N**	**%**
Patient communication/information		
In my hospital, physicians do not...		
... provide written information about diagnostics and/or therapy	5	22
... provide information about patient associations	10	44
Emphatic contact		
In my hospital, most physicians do not...		
... listen carefully to their patient	4	16
... show personal interest in their patient	7	28
... show attention for emotions and coping of the patients' relatives	8	35
(III) Patients	**N**	**%**
In my hospital...		
... patients may not always participate in decision-making	3	13
(IV) Organisational context	**N**	**%**
Waiting times		
In my hospital...		
... time between referral and first diagnostics was >10 weekdays	5	19
... time between first diagnostics and final diagnosis was >15 weekdays	9	35
... treatment could not be started as soon as possible after diagnosis	8	31
... hospital appointments for diagnostics/therapy were not planned on one day	13	50
... my own physician is not available in case of urgent problems	4	15
(V) Social context	**N**	**%**
Teamwork and personalised care		
In my hospital...		
... physicians are not informed about agreements made with other physicians	4	17
... there is no central contact person for making appointments	5	22
... patients often see different physicians for diagnostics and treatment	4	17
Guidance and support		
In my hospital...		
... no help was offered for dealing with emotions and practical problems	8	35
... no psychological help was offered after breaking bad news	9	39

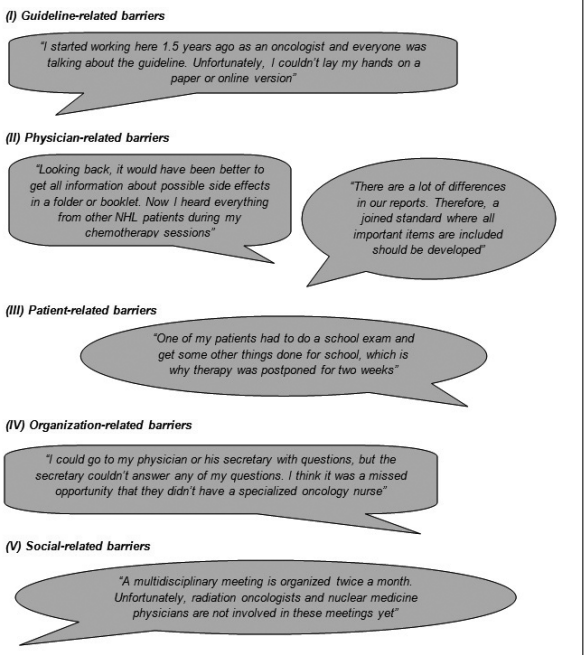
[a] **Not all questions were answered by all patients. Therefore the results do not always relate to the total study population (n=28).**

Table 2b. *Most important perceived barriers that influence daily NHL care according to physicians*

Perceived barriers per domain	Number of physicians[a]	
(I) Guideline	**N**	**%**
The NHL guideline...		
... is not really known to me	27	24
... is not easily accessible for me	35	32
... is not extensively read by me	35	32
... is not used as a reference	46	42
... does not give enough room for including patient preferences	18	16
... is not clear enough for my profession	35	32
... is not up-to-date for my profession	71	66
... is hard to update because of lack of consensus	34	31
... *should be updated*	72	66
... *should be available online*	91	83
A local NHL protocol...		
... is not available at our hospital	52	47
... is not clear enough for my profession	9	15
... is not up-to-date for my profession	13	22
... *should be available online*	35	63
... *is not necessary because we use protocols of other hospitals*	29	58
(II) Physicians	**N**	**%**
Working according to the NHL guideline/protocol		
In our hospital...		
... the IPI score is not routinely calculated for NHL	6	15
... indicator lesions are not routinely measured	14	21
... the Cheson response criteria are not routinely used	22	40
Standardised forms		
In our hospital no standardised forms		
... for pathology requests are available	41	70
... for pathology reports are available	32	56
... for radiology/nuclear medicine requests are available	39	59
... for radiology/nuclear medicine reports are available	43	65
... no integrated reports are accomplished for radiology and nuclear medicine	20	32
(III) Patients	**N**	**%**
-		
(IV) Organisational context	**N**	**%**
Materials and facilities		
In our hospital...		
... no standard NHL patient information is available	13	22
... no standard procedure for after care is available	9	15
... no compulsory training days for NHL care are established	34	32
... no specialised oncology nurse is present	15	24
Waiting times		
In our hospital...		
... diagnostics can usually not be done in 15 weekdays	9	16
(V) Social context	**N**	**%**
Multidisciplinary meetings...		
... do not include all professions involved	53	56
... *with all professions involved would improve NHL care*	52	64

[a] **Not all questions are applicable to all five professions or are answered by all physicians. Therefore the results do not always relate to the total study population (n=132).** [b] **Based on multiple choice questions where more than one answer could be checked. Items in italics are perceived as facilitators.**

Figure 1. *Illustrative quotations from patients and physicians concerning barriers of quality of NHL care delivered*



an up-to-date, online version of the guideline (66% and 83%, respectively) and an online version of the local NHL protocol (63%). Of note, 24% of the physicians were not familiar with the guideline.

(II) Physician-related barriers. The most frequently mentioned barrier by patients concerned physicians providing insufficient information about patient associations (44%), the lack of attention for coping of the patients' relatives (35%) and not showing personal interest in a patient (28%). Insufficient written information provided by physicians was mentioned frequently during the interviews and 22% agreed on this in the survey.
Physicians did not mention barriers regarding information provision and communication, but mainly focused on the lack of standardised forms for diagnostic requests and reports (ranging from 56-70%) and the not routinely used Cheson response criteria (40%).

(III) Patient-related barriers. Concerning patient self-reflection, some patients mentioned a lack of participation in decision-making (13%); physicians did not mention barriers in this domain. In the interviews, a few patients and physicians mentioned patients' preferences concerning postponement of diagnostics or treatment as impeding factor.

(IV) Organisation-related barriers. Patients pointed out waiting times as a barrier, with hospital appointments not planned on one day (50%) being the most common issue. Physicians mentioned the lack of compulsory training days for NHL care (32%) and the absence of a specialised oncology nurse (24%) as a barrier. The latter was also mentioned frequently during the interviews.

(V) Social-related barriers. Barriers perceived by patients included items on teamwork and guidance & support; for example, the lack of help offered after breaking bad news (39%). Physicians pointed out barriers concerning structural multidisciplinary meetings. Especially, the lack of participation of all involved disciplines in the multidisciplinary meetings (56%) was seen as a barrier. They agreed that the involvement of all disciplines participating in NHL care in multidisciplinary meetings would improve NHL care (64%).

Differences in perceived barriers between the disciplines involved

Significant differences ($p<0.05$) in perceived barriers between the four disciplines involved in NHL care are found (*table 3*). Pathologists more often perceived barriers according to accessibility of the guideline, (57%) its use (52%), and clarity of a local protocol (63%) compared with other disciplines; radiation oncologists perceived these barriers in only 14%, 14% and 0%, correspondingly. Regarding standardised forms, haemato-oncologists less often perceived barriers concerning standardised pathology forms (42%) than pathologists (84%) and 21% of the haemato-oncologists perceived the lack of integrated forms as a barrier compared with 48% of the radiologists/ nuclear medicine physicians. In the organisational context, standardised patient information about NHL seems less often available for radiation oncologists than haemato-oncologists (classified as barrier in 50% versus 15%) and the absence of compulsory training days for NHL was most rated as barrier by radiation oncologists (71%).

DISCUSSION

This is the first study to identify barriers for delivering good quality of care to NHL patients. The interviews and survey showed considerable differences in focus between patients and physicians involved in NHL care. Patients pointed out more barriers regarding patient communication, guidance and waiting times, whereas physicians focused on guideline-related barriers and standardisation of forms and procedures. Among the physicians from the four disciplines involved in NHL care significant differences were encountered in lack of guideline use, standardised forms, patient information and compulsory training days.

Table 3. *Differences in perceived barriers between physicians of the disciplines involved in NHL care*

Perceived barriers per domain	Haemato-oncologists		Pathologists		Radiologists/ nuclear med. physicians		Radiation oncologists		*P* value
(I) Guideline	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	
The NHL guideline...									
... is not easily accessible for me	11	24	12	57	10	33	2	14	0.02
... is not extensively read by me	9	20	11	52	13	43	2	14	0.01
... is not up-to-date for my profession	34	74	7	35	22	73	9	64	0.01
... *should be updated*	32	71	10	50	17	57	13	93	0.04
A local NHL protocol...									
... is not clear enough for my profession	3	13	5	63	1	7	0	0	0.00
(II) Physicians									
Standardised forms									
In our hospital...									
... no standardised forms for pathology reports are available	16	42	16	84	n.a.	n.a.	n.a.	n.a.	0.00
... no integrated reports are accomplished for radiology and nuclear medicine	8	21	n.a.	n.a.	12	48	n.a.	n.a.	0.03
(IV) Organisational context									
Materials and facilities									
In our hospital...									
... no standard NHL patient information is available	7	15	n.a.	n.a.	n.a.	n.a.	6	50	0.01
... no compulsory training days for NHL care are established	11	23	5	26	8	31	10	71	0.01

n.a. = not applicable to this profession.

In this study, an important barrier pointed out by patients was the need for clear information from physicians. Studies in other areas of healthcare have also pointed out the lack of information provided by physicians.[18,19] However, the ability of patients to recall the information provided might not always be optimal as well.[20] Next to good communication, emphatic contact is often put forward when experienced care is evaluated. Our results and those of other studies[21,22] show that these two topics are important issues of concern for patients. Physicians, on the other hand, did not pay special attention to barriers regarding information provision and communication to patients. Research shows, however, that emphatic communication can influence patient satisfaction, quality of life and even medical outcome.[23,24]

Interestingly, a barrier frequently mentioned by physicians concerned the lack of an up-to-date guideline. In general, clinical guidelines aim to promote evidence-based practice and improve patient outcomes.[25] It is hence necessary to update guidelines on a regular basis. The Dutch NHL guideline[6] was developed in 2004 and has not been updated since. However, several web-based protocols were initiated[26] and international evidence-based guidelines are available online.[5,7] The use of such protocols is associated with better patient outcomes.[27,28] We therefore believe that the implementation of an updated national NHL guideline could help to reduce perceived barriers and may result in improved quality of NHL care.

In several cancer studies guideline adherence was associated with better overall survival or progression-free survival.[29,30] Our study showed several barriers resulting from not working according to guidelines, for example lack of assessing the Cheson therapy response criteria (*table 2b*). Recently, a national imaging working group developed recommendations for the standardisation of PET-CT scan requests and reports.[31] Another important barrier is the lack of well-organised multidisciplinary meetings. Guidelines for optimal functioning of multidisciplinary meetings have recently been formulated.[32] The dissemination of these recommendations is definitely an important step towards improved care.

With regard to the strengths of this study, a unique setting was created to obtain a broad overview of perceived barriers in current NHL care. First of all, physicians of all disciplines involved in NHL care were approached to participate in our study. This gave us the opportunity to compare barriers perceived between the four disciplines involved. Our results indicate differences in perceived barriers among physicians involved in NHL care, which is valuable for subsequent research.

Second, we incorporated the patients' perspective in the study. The inclusion of NHL patients in our study is in line with the increasingly important role of patients in managing their own hospital care. This study clearly shows the added value of incorporating patients' points

of view: barriers concerning patient communication and information provision were not experienced by physicians, whereas this was a main concern for patients. To our knowledge, this is the first study that looks at such a wide-ranging study population, including patients as well as physicians from all disciplines involved.
Third, identification of the barriers perceived was based on both qualitative and quantitative research. Interviews were used to qualitatively explore barriers perceived by patients and physicians, after which these results were quantified in a survey. This thorough overview is not only applicable on a national level, but might also be valuable internationally since recommendations on NHL care in the Dutch guidelines and protocols largely conform to international guidelines.[4-7,26]

There are also some limitations in this study that should be addressed. First, we were not able to calculate the survey participation rates. LVN and the Dutch Societies do not provide home addresses of patients or physicians for study purposes and there is no registration for physicians specialised in NHL care. Based on these restrictions, we do believe the best possible way to approach participants was utilised.
Second, the recruitment method used is possibly also responsible for the relatively low number of responses and could have introduced underreporting. We think that highly motivated persons might participate more often in research than less motivated/involved ones. For example, the percentage of physicians who do not really know the content of the NHL guideline might be even lower in the non-responder group. Another possible explanation for the low number of responses might be that NHL patients are often older patients who may not be familiar with the Internet. Our responders, however, represented NHL patients and physicians of all age groups and our total study population was diverse (*table 1*).
Third, the surveys applied were not validated before use. However, to ensure that they truly represent the complete spectrum of NHL care, the surveys were based on the barriers identified in the prior interviews. In addition to this, the patient survey was derived from the standardised CQI questionnaire for cancer patients. For this reason four answer possibilities were used in the patient survey, instead of the frequently used five-point Likert scale. To the best of our knowledge, no validated questionnaires were available that could have been used in our study.

In conclusion, this study gives a broad overview of barriers that influence NHL practice, as perceived by patients and physicians. Barriers most mentioned by patients were lack of guidance & support, long waiting times and lack of clear communication and emphatic contact. Physicians most often stated lack of an up-to-date, online NHL guideline, lack of standardised forms for diagnostics and the absence of multidisciplinary meetings with all physicians involved. Among the four disciplines involved in NHL care significant differences were encountered in guideline use and the lack of standardised forms, patient information and compulsory training days. Together with the gaps found in quality of care by Wennekes *et al.*,[12] our results form a solid basis to develop a tailored implementation strategy to increase the quality of NHL care and to test this strategy on effectiveness and costs.

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SPECIAL ARTICLE

Severe hypophosphataemia after intravenous iron administration

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ABSTRACT

Currently, in many centres, intravenous administration of iron is becoming increasingly popular because of higher efficacy and decreased side effects, mainly gastrointestinal, compared with oral iron therapy. Studies of intravenous ferric carboxymaltose administration in the postpartum setting and in patients with non-dialysis-dependent chronic kidney disease revealed a decrease in serum phosphate levels that was generally asymptomatic and transient.

Here, we report four cases of severe and symptomatic hypophosphataemia after intravenous iron administration. All patients received this as therapy for iron deficiency anaemia due to heavy menstrual bleeding. In most cases, a pre-existent disorder in the phosphate homeostasis existed, such as a secondary (cases 3 and 4) or tertiary hyperparathyroidism (case 1). However, in the second case there were no risk factors for a dysregulation of the phosphate homeostasis.

Based on these findings, we conclude that severe and symptomatic hypophosphatemia can occur as a side effect of intravenous iron administration and can persist for months after administration. Especially patients with low phosphate levels prior to therapy due to concomitant disorders in phosphate homeostasis (e.g. hyperparathyroidism, vitamin D deficiency) are at risk.

KEYWORDS

Adverse effects, ferric compounds, hypophosphaetemia, intravenous iron administration/supplementation

What was known on this topic?
Intravenous iron supplements are highly effective in treating iron deficiency anaemia. Besides a transient, asymptomatic hypophosphataemia, there are few adverse effects of these intravenous preparations compared with oral iron supplements.

What does this add?
This article emphasises the importance of determining pre-existent disorders in phosphate homeostasis and continuous monitoring of serum phosphate levels after intravenous ferric carboxymaltose administration as the provoked hypophosphataemia can be severe, prolonged and potentially life-threatening.

INTRODUCTION

Iron deficiency is one of the most common causes of anaemia and is frequently encountered in patients with chronic kidney disease. Besides investigating the cause of the deficiency, symptoms can be relieved by iron supplements, which can be either orally or intravenously administered. Oral iron supplementation is often poorly tolerated due to a high rate of gastrointestinal side effects, which leads to dose reduction or non-adherence to treatment.[1-3] Recently, intravenous iron supplements were developed that allow administration of large amounts of iron by one simple infusion. These iron supplements are more effective in achieving haemoglobin level increases and have less drug-related adverse effects.[4]

However, some studies of intravenous ferric carboxymaltose administration reported a decrease in serum phosphate levels. The induced hypophosphataemia was generally asymptomatic and transient.[5-8] In non-dialysis dependent chronic kidney patients, serum phosphate levels decreased in 3.8% of the patients receiving intravenous iron supplementation and did not reach a clinically important level.[8] However, in this paper it is demonstrated that a severe and potentially

life-threatening hypophosphataemia can be provoked by intravenous administration of iron supplements.

CASE REPORT

Case 1

A 45-year-old female of Asian origin received an unrelated living donor kidney allograft in May 2008 for end-stage renal failure due to diabetes mellitus type I. The postoperative course was complicated by a delayed graft function. Four years later, she has a good transplant function (estimated glomerular filtration rate (eGFR) of 60 ml/min/1.73 m^2) on a dual immunosuppressive regimen of tacrolimus (trough levels 5.5-10.5 µg/l) and mycophenolate mofetil (500 mg twice daily) and adequate glycaemic control with a basal-bolus insulin regimen (HbA1c 55 mmol/mmol). To treat the vitamin D deficiency, she received colecalciferol 400 IE, which resulted in sufficient 25(OH)vitamin D levels (78 nmol/l, normal 50-150 nmol/l). However, the pre-transplant secondary hyperparathyroidism did not resolve completely over time with serum parathyroid hormone (PTH) levels remaining around 12.0 pmol/l (normal 1.0-8.0 pmol/l) and near-normal serum phosphate (P) levels between 1.37 and 0.70 mmol/l (normal 0.80-1.40 mmol/l). Furthermore, mild systolic hypertension remained which was treated with a combination of amlodipine 10 mg once daily and irbesartan 150 mg twice daily.

Due to a benign endometrial polyp, the patient suffered from heavy menstrual bleeding which led to the development of iron deficiency anaemia. Laboratory findings were: Hb 6.0 mmol/l (normal 7.5-10.0 mmol/l), serum iron 4 µmol/l (normal 10-25 µmol/l), serum transferrin 84 µmolFe/l (normal 35-65 µmolFe/l), serum iron saturation 5% (normal 20-45%), serum ferritin 5 µg/l (normal 8-252 µg/l). As oral iron supplements caused significant gastrointestinal side effects, she received a single infusion of 1000 mg ferric carboxymaltose (Ferinject®, Vifor Nederland BV, the Netherlands). Eight days after initiating therapy, the patient developed generalised weakness and nausea. A deep hypophosphataemia was diagnosed (P 0.25 mmol/l) and inappropriate phosphaturia. The fractional excretion of phosphate was 59%; with hypophosphataemia it should be below 5%. There were no cardiopulmonary or haematological manifestations. The patient was admitted to hospital and received 40 ml sodium glycerophosphate intravenously (1 ml = 1 mmol P, Glycophos®, Fresenius Kabi Nederland BV, the Netherlands). Forty-eight hours later, the serum phosphate level rose to 0.43 mmol/l and her clinical condition improved rapidly. She was discharged with instructions to continue oral phosphate supplementation by sodium glycerophosphate 20 ml three times a day. Seventy-two hours after discharge, the patient returned to the emergency department presenting with vertigo, nausea, diarrhoea, general weakness and tingling in both hands. Again, profound hypophosphataemia (P 0.17 mmol/l) and continued inappropriate phosphate fractional excretion of 33.3% were found. Moreover, plasma levels of fibroblast growth factor 23 (FGF23) were elevated at 202 RU/ml (normal <125 RU/ml). The patient was re-admitted to the hospital for short-term intravenous phosphate repletion, sodium glycerophosphate 20 ml twice daily, and within one day the serum phosphate level rose to 0.55 mmol/l. As the symptomatic hypophosphataemia resolved, treatment was continued with oral phosphate repletion (1 ml = 67 mg P), 20 ml three times a day. The patient was discharged with stable serum phosphate levels and within six weeks oral phosphate repletion was ceased. On subsequent controls, the serum phosphate levels remained stable within the normal range.

Case 2

A 42-year-old Caucasian female with known systemic lupus erythematosus since 1998 developed iron deficiency anaemia (Hb 7.4 mmol/l, serum iron 5 µmol/l, serum transferrin 68 µmolFe/l, serum ferritin 15 µg/l) due to heavy menstrual bleeding, while on anticoagulant therapy consisting of calcium carbasalate and acenocoumarol for antiphospholipid syndrome and repeated cerebrovascular accidents. On ultrasound, a persistent ovarian follicle and endometrial hyperplasia were diagnosed. Initially, oral iron supplements were prescribed to treat the iron deficiency anaemia. However, the patient developed profound side effects that led to discontinuation of oral treatment and subsequent administration of three infusions of 1000 mg ferric carboxymaltose with a six-week interval. Three days after the last infusion, the patient was diagnosed with deep hypophosphataemia (P 0.32 mmol/l) and inadequate fractional excretion of phosphate (12.4%). Symptoms attributable to hypophosphataemia were not present. Additional laboratory findings revealed normal values of serum parathyroid hormone (4.92 pmol/l) and 25(OH) vitamin D (97 nmol/l) and resolved anaemia (Hb 8.6 mmol/l). The patient was advised to increase her dietary phosphate intake by increasing the intake of protein-enriched meals and the phosphate levels were restored to normal levels within four weeks. However, due to continued heavy menstrual bleeding, the iron deficiency anaemia recurred (Hb 4.5 mmol/l). Upon this, the patient was treated with a single infusion of 100 mg iron sucrose (Venofer®, Vifor Nederland BV, the Netherlands). After eight days, the patient once more developed a deep, although again asymptomatic, hypophosphataemia (0.33 mmol/l), which was resolved in a similar way by increasing the dietary intake of phosphate.

Case 3

A 33-year-old Hindu female underwent a laparoscopic Roux-Y gastric bypass in 2010 on account of obesity

(body mass index 44 kg/m²). The postoperative course was complicated by pneumonia, which was treated with amoxicillin-clavulate and ciprofloxacin, and one year later she underwent a laparoscopic cholecystectomy because of symptomatic gallstones. During the following two years, she lost 55 kg in weight. In February 2012, she developed anaemia due to heavy menstrual bleeding (Hb 5.8 mmol/l, MCV 72 fl, ferritin 2 µg/l, transferrin saturation 5%). In addition, vitamin D deficiency (25(OH) vitamin D 13 nmol/l) and secondary hyperparathyroidism (PTH 12.8 pmol/l) were diagnosed. She was treated for three months with sustained-release ferrous sulphate 287 mg once daily, calcium 1000 mg and colecalciferol 800 IU. Seven months later, she presented to the internal medicine outpatient clinic with persisting anaemia (Hb 5.2 mmol/l, MCV 64 fl, ferritin 1 µg/l, transferrin saturation 1%). The vitamin D deficiency and hyperparathyroidism were also still present (25(OH) vitamin D 28 nmol/l, PTH 16.7 pmol/l). In addition, she had vitamin B12 deficiency (vitamin B12 <111 pmmol/l, normal 142-725 pmol/l). Her renal function was good with an eGFR above 90 ml/min/1.73 m². The persistent iron deficiency anaemia was treated with two infusions of 1000 mg ferric carboxymaltose with a one-month interval. The vitamin D deficiency was treated with oral colecalciferol 50,000 IU monthly and the vitamin B12 deficiency with six intramuscular injections of 1 mg hydroxocobalamin. Three weeks after the last ferric carboxymaltose infusion, routine laboratory control revealed severe hypophosphataemia (P 0.22 mmol/l). A week before admission she had suffered from vomiting and diarrhoea, but two days before admission these complaints had passed spontaneously. Upon laboratory testing, the anaemia had resolved (Hb 7.1 mmol/l, ferritin 300 µg/l, vitamin B12 226 pmol/l). Other laboratory results were: calcium 2.25 mmol/l, albumin 39 g/l, 25(OH) vitamin D 62 nmol/l, magnesium 0.71 mmol/l, parathyroid hormone (PTH) 24.8 pmol/l and fractional excretion of P 23.7%. During a ten-day admission, she was treated with intravenous sodium glycerophosphate 40 ml three times daily in combination with 15-20 ml oral phosphate solution (1 ml = 67 mg P) once daily. Restoration of phosphate levels to normal levels was hard to achieve. After ten days, she was discharged with a phosphate level of 0.46 mol/l, which increased to 0.53 mmol/l nine days after discharge with 15 ml oral phosphate supplementation once daily. Two months later, the serum phosphate level had almost returned to normal limits (P 0.70 mmol/l).

Case 4

A 47-year-old female of Caribbean origin with known Graves' hyperthyroidism since 1997 was treated medically until 2003. Then, she received radioactive iodine therapy, after which her thyroid function normalised. In December 2012, she was admitted because of severe anaemia (Hb 3.3 mol/l, MCV 54 fl, ferritin 2 µg/l, transferrin saturation 3 %). She presented with increased menstrual bleeding due to a small intramural fibroid, fatigue, headache and palpitations. Additionally, she had hypothyroidism (TSH 22.4 mU/l, normal 0.4-4.0 mU/l, free thyroxine 8.8 pmol/l, normal 10.0-24.0 pmol/l). Calcium (2.25 mmol/l), phosphate (0.85 mmol/l), albumin (37 g/l) and kidney function (eGFR >90 ml/min/1.73 m²) were normal. Besides oral levothyroxine 50 µg once daily and three units of red blood cell transfusions, she received three gifts of 1000 mg ferric carboxymaltose with a two-week interval between the first two infusions and a one-month interval between the second and third. Four days after the last infusion, routine laboratory control revealed a severe hypophosphataemia (P 0.28 mmol/l). The hypothyroidism had improved (TSH 12.8 mU/l, free thyroxine 10.2 pmol/l) and the anaemia had resolved (Hb 8.9 mmol/l, ferritin 1646 µg/l, transferrin saturation 56 %). Other laboratory results were: calcium 2.25 mmol/l, albumin 38 g/l, magnesium 0.74 mmol/l, 25(OH) vitamin D 49 nmol/l, PTH 9.8 pmol/l and a fractional excretion of P 28.4%. Besides feelings of depression, she had no complaints. She was treated with intravenous sodium glycerophosphate 40 ml and oral phosphate solution (1 ml = 67 mg phosphate) 15 ml three times daily. Serum phosphate increased to 0.50 mmol/l. She was discharged with continuation of oral phosphate solution.

Six weeks afterwards, routine laboratory control again revealed severe hypophosphataemia (P 0.20 mmol/l). She complained of weakness and fatigue and the hypothyroidism had worsened (TSH 30.8 mU/l and free thyroxine 8.3 pmol/l). The level of haemoglobin remained normal. She was treated with 40 ml intravenous sodium glycerophosphate, with an additional 20 ml the next day. Serum phosphate increased to 0.89 mmol/l. Levothyroxine was increased to 100 µg daily.

Six days later, once again, the patient developed hypophosphataemia (P 0.33 mmol/l). Fractional urinary excretion of phosphate was 18%. Once more, she received 20 ml intravenous sodium glycerophosphate for two days, upon which the serum phosphate levels increased to 0.87 mmol/l. In addition, 25(OH) vitamin D was 45 nmol/l with normal levels of serum calcium and magnesium. Unfortunately, the PTH was not determined at that point. The day after admission serum FGF23 level was 119 RU/ml. She was discharged on oral phosphate solution 20 ml three times daily (1 ml = 67 mg phosphate) and colecalciferol 50,000 IU/ml weekly.

In the following month, her serum phosphate levels still remained low with values between 0.41 and 0.63 mmol/l. Due to the worsened hypothyroidism (TSH 72.8 mU/l), compliance of intake of oral phosphate solution seemed disputable. However, the vitamin D deficiency had resolved (25(OH) vitamin D 64 nmol/l) and afterwards the phosphate levels normalised to levels between 0.74 and 0.81 mmol/l.

DISCUSSION

Many studies show that intravenous ferric carboxymaltose is superior to oral iron supplementation in restoring Hb levels.[1,3,4,8] Furthermore, it has less drug-related adverse effects.[8] However, some clinical studies found a transient, generally non-symptomatic, hypophosphataemia after intravenous iron supplementation. In this paper, we report four cases of severe and symptomatic hypophosphataemia. Despite the fact that concomitant disorders of phosphate homeostasis were present in cases 1, 3 and 4, the serum phosphate levels of all patients were normal or near-normal prior to intravenous iron administration. Therefore, we conclude that the cause of hypophosphataemia must be intravenous iron administration and that hypophosphataemia provoked by intravenous iron supplementation can be severe, symptomatic and prolonged.

Phosphate homeostasis is maintained via the bone-kidney endocrine axis, in which PTH, vitamin D, and FGF23 are important regulators. Renal phosphate excretion is regulated mainly by PTH and FGF23, which are both phosphaturic hormones.[9-11] Hyperparathyroidism was present in cases 1, 3, and 4. In the last two cases this can be attributed to vitamin D deficiency, since calcium was at the lower level of the normal range and renal function was normal. In addition, it seems unlikely that the degree of the hypovitaminosis D, with only a slight upregulation of PTH, can explain the deep hypophosphataemia by itself. Also, all these patients had increased urinary phosphate excretion that was inadequate to the degree of the hypophosphataemia. The inadequate phosphate excretion that was seen could be explained by hyperparathyroidism, but this was not present in case 2. Thus, other mechanisms have to be involved.

Studies have shown that hypophosphataemia induced by intravenous iron administration is mediated by an increase in serum levels of FGF23.[12] In our first case, this was indeed demonstrated, as FGF23 levels and renal phosphate excretion were increased during the second episode of hypophosphataemia after intravenous iron administration. The fourth case showed FGF23 levels at the upper limit of the normal range two months after the last infusion of ferric carboxymaltose. It may be speculated that the level of FGF23 might have been higher in between. On the other hand, the level of FGF23 can be regarded as inadequately high in relation to the degree of the hypophosphataemia.

Furthermore, it is interesting to note that in the third case, the level of PTH increased while the vitamin D level normalised. Although levels of FGF23 and calcium were not determined in that case, the increase in PTH could be explained by an increase in FGF23 levels. Studies have shown that the increase in PTH in patients with chronic kidney disease is mediated by FGF23. As FGF23 suppresses the synthesis of calcitriol, it indirectly stimulates PTH secretion.[13,14] Although FGF23 inhibits PTH secretion in the short term, with continuous stimulation (e.g. during chronic kidney disease) the inhibiting effect of FGF23 on PTH fades away and levels of PTH increase, possibly by downregulation of the FGF23/Klotho receptor complex.[15-17] Thus, the increase in PTH in case 3 may be explained by sustained increase of FGF23 levels following intravenous iron administration.

The FGF23-mediated phosphaturia might also explain the difference in the incidence of hypophosphataemia after ferric carboxymaltose administration between patient populations. In patients with chronic kidney disease, hypophosphataemia is observed in 3.8% of all cases, while in iron deficiency anaemia due to gynaecological diseases, this percentage rises to 70%.[5,8] As patients with chronic kidney disease already have a restricted renal excretion of phosphate and upregulated levels of FGF23, a further increase in FGF23 will have a mild effect on their renal phosphate excretion.[18,19] In patients with a normal glomerular filtration rate, the effect can be much more pronounced as is shown in this report. In particular, when phosphate levels are already relatively low prior to treatment, which can be the case after renal transplantation due to persistent hyperparathyroidism (case 1) or by concomitant vitamin D deficiency (cases 3 and 4), an increase in FGF23 levels may result in a significant hypophosphataemia.

In addition, recent studies suggest that iron deficiency stimulates FGF23 transcription in osteocytes, after which excess FGF23 is cleaved within the osteocytes into inactive C-terminal FGF23 (cFGF23). This leads to a stable level of active, intact FGF23 (iFGF23).[20,21] Administration of ferric carboxymaltose seems to disrupt this balance by inhibiting the cleavage of iFGF23. Conversely, iron dextran does not have an effect on FGF23 cleavage.[20] These findings may clarify the difference in incidence of hypophosphataemia after administration of different intravenous iron supplements, though the mechanism within the osteocytes remains unclear. In the second case, however, hypophosphataemia occurred to the same degree after both administration of ferric carboxymaltose and iron sucrose. Therefore, development of hypophosphataemia should be considered after administration of different types of intravenous iron preparations.

Finally, it is important to note that intravenous and oral phosphate supplementation may be harmful. It implies a risk of hypocalcaemia, arrhythmias, ectopic calcification, and can induce acute phosphate nephropathy.[22,23] In addition, although the nadir of serum phosphate is usually reached two weeks after intravenous iron administration, this time course can be prolonged.[5-7] Suppression of 1α-hydroxylation of vitamin D, which is one of the effects of FGF23, can last up to three months after the last administration.[24] In the fourth case, hypophosphataemia persisted for up to three months, which may be explained

by this mechanism. Therefore, physicians should be aware of possible adverse effects of phosphate supplementation and a prolonged and recurrent hypophosphataemia.

In order to prevent this potentially life-threatening side effect of intravenous iron administration, we propose routine evaluations of the risk of development of hypophosphatemia prior to treatment with ferric carboxymaltose. Blood analysis should include serum levels of creatinine and phosphate. In patients with a compromised phosphate homeostasis (e.g. patients with a good renal function and low or on the lower end of the normal range serum phosphate), it is advised to measure serum phosphate once more two weeks after commencing treatment, when the phosphate levels reach a nadir. In addition, alternative iron preparations (such as iron dextran) should be considered. However, these imply an increased risk of other side effects, such as allergic reactions in the case of iron dextran.[25]

CONCLUSION

Clinicians should be aware of this potential complication and carefully evaluate indications for intravenous iron supplementation. Evaluation of pre-existent levels of serum phosphate and renal function is warranted to evaluate the risk of developing hypophosphataemia. In addition, monitoring of phosphate balance should be performed, especially in patients susceptible to a dysregulation of the phosphate homeostasis (e.g. patients with hyperparathyroidism, hypovitaminosis D, pre-existent hypophosphataemia in combination with good renal function). Further research is required to determine whether lower doses of intravenous ferric carboxymaltose or other intravenous iron preparations may be preferable in this patient population.

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SPECIAL REPORT

Treatment of hyperglycaemia in diabetic ketoacidosis: natura non facit saltus

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ABSTRACT

In the treatment of severe diabetic ketoacidosis the gradual correction of glucose, electrolyte and fluid derangements is of utmost importance. In this paper the authors provide practical recommendations for these corrections based on novel pathophysiological insights.

KEYWORDS

Diabetic ketoacidosis, hyperglycaemia, hyponatraemia

INTRODUCTION

One of the most challenging parts of the treatment of severe diabetic ketoacidosis (DKA) is to gradually correct the glucose, sodium and fluid disturbances. Osmotic shifts before and even more so during treatment play a crucial role in the development of cerebral oedema, the most dreaded complication of DKA. Although it only seldom occurs in adults, due to its often devastating clinical course with a high mortality (25%) and severe neurological sequelae (35-40%), a substantial part of the treatment is focussed on its prevention.

Several guidelines provide recommendations with regard to glucose, electrolyte and fluid management. Nevertheless, in daily practice treatment of patients with severe DKA continues to generate a lot of discussion. The main topics are:

- What is the optimal rate of glucose correction?
- What is the main parameter that should be used to guide therapy?
- What increase in sodium is acceptable?

Therefore, in this special report, we will illustrate this problem with one case history, provide some pathophysiological background and discuss these issues.

CASE

A 51-year-old woman presented to the emergency department with polydipsia, polyuria, vomiting and diarrhoea. She had lost 6 kg of weight in one week. Her medical history was unremarkable and she was not on any medication. Physical examination revealed a somnolent patient with Kussmaul breathing, a blood pressure of 97/76 mmHg and a pulse of 75/min. Her laboratory results are presented in *table 1*. She had severe acidaemia, due to a combination of ketoacidosis, lactic acidosis and renal failure. Her severe hyponatraemia was probably caused by hyperglycaemia and volume depletion. Furthermore, in the days before admission the patient had drunk large amounts of glucose-containing fluids and water.

The physician at the emergency department had started suppletion with isotonic fluids (NaCl 0.9%), and administered a bolus of insulin, followed by continuous insulin infusion. She was admitted to the intensive care unit. *Figure 1* shows the course of the glucose concentration and the effective serum osmolality. After three hours insulin administration was reduced and the isotonic fluid (NaCl 0.9%) was switched to hypotonic fluid (NaCl 0.65%). In the next 24 hours the serum glucose, electrolytes and anion gap normalised rapidly and the acidaemia disappeared. She felt better and was transferred to the department of internal medicine.

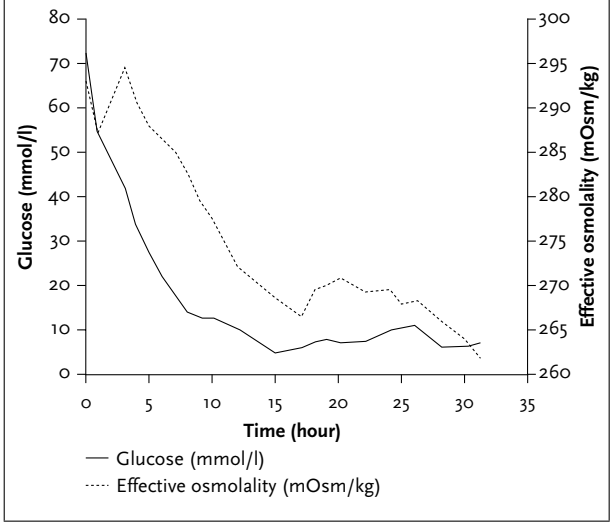
DISCUSSION

The glucose concentration of our patient decreased very quickly, 30 mmol/l in three hours. This was compensated by an increase in sodium concentration (19 mmol/l in three hours), which kept the effective serum osmolality more or less equal (292 mosm/kg and 294 mosm/kg).[1] However, the treating physician was anxious about the fast decrease of glucose and rapid increase of sodium. The

Table 1. *Laboratory results*

Laboratory results	
Haemoglobin	9.1 mmol/l
Thrombocytes	381 x 10^9/l
Leukocytes	23.3 x 10^9/l
CRP	5 mg/l
Glucose	72.3 mmol/l
Sodium	103 mmol/l
Potassium	7.4 mmol/l
Chloride	69 mmol/l
Lactate	4.5 mmol/l
Creatinine	261 µmol/l
Albumin	32 g/l
Anion gap	32 mmol/l
Arterial blood gas analysis	
pH	6.90
pCO_2	12 mmHg
pO_2	70 mmHg
HCO_3^-	2.4 mmol/l
Base excess	-28.5 mmol/l
O_2 saturation	87%
Urine	
Ketones	+++

Figure 1. *Glucose concentration and effective serum osmolality. Effective osmolality = 2 x [sodium + potassium] + [glucose]*



isotonic fluids were changed to hypotonic fluids. After 18 hours the effective serum osmolality had dropped to 270 mosm/kg. Was this reflex appropriate? Before we try to answer the main topics of discussion as formulated in the introduction, we will first provide some pathophysiological background on the osmotic shifts of severe DKA that occur before and during treatment.

Pathophysiology osmotic shifts

Osmoles and the blood

Osmolality is defined as the number of moles of a compound that contribute to the osmotic pressure of a solution. Osmolality in serum is predominantly determined by electrolytes (Na^+, K^+ and Cl^-), urea and glucose. A difference in osmolality on both sides of a membrane will cause a shift of water until an equilibrium has been reached. If an osmole can cross the membrane freely, a change in its concentration will not cause a shift of water. It is not an 'effective' osmole, such as for example urea and, in the presence of insulin, glucose. The uptake of glucose in the cells is facilitated by insulin dependent glucose transporters (GLUT 4). However, in the absence of insulin this becomes impossible, and glucose will be an effective osmole. Therefore, in the presence of hyperglycaemia, due to osmosis, water will leave the cell, resulting in intracellular dehydration. Extracellularly, the – relative – water excess will decrease the sodium concentration. In healthy subjects, for every 2.5 mmol/l rise in serum glucose concentration, the serum sodium concentration will decrease by 1 mmol/l.[2]

Glucose and the brain

In the brain, glucose will first have to pass the blood-brain barrier. This transport takes place by facilitated diffusion. GLUT 1 receptors at the endothelial surface facilitate this transport, independent of insulin.[3] However, this transport is not at all complete and is not very fast. In animal studies and the scarce human studies, up until a serum glucose of 30 mmol/l, the relationship between serum and cerebrospinal fluid (CSF) glucose concentrations is linear, the concentration in the CSF being roughly 1/3 of the serum concentration, reaching a nadir of 0 mmol/l when serum glucose is 2.2 mmol/l.[4] Above a serum glucose of 30 mmol/l, some data suggest even less efficient transport of glucose.[3]

Next, glucose has to enter the brain cell facilitated by GLUT 3 receptors. It is not entirely clear whether this transport is limited as well. The glucose gradient at the blood-brain barrier, however, is most important, and causes a shift of water from the CSF to the intravascular compartment during hyperglycaemia.

In animal experiments, brain water decreased during acute hyperglycaemia, with an increase in brain sodium concentration.[5] As in hypernatraemia, cerebral cell shrinking stimulates the formation of osmolytes such as glutamine, glutamate and inositol. The concomitant increase in intracellular osmolality attracts water, resulting in restoration of cellular volume.

The brain during correction of hyperglycaemia

When therapy is initiated with volume resuscitation and insulin, glucose concentration in serum will decrease rapidly. Together with water, glucose enters the cell, resulting in an increase of the serum sodium concentration. When, due to volume repletion, diuresis ensues, this will initially lead to a loss of mostly glucose and water, thereby lowering the serum glucose and increasing serum sodium concentration.

Altogether, serum osmolality will decrease gradually. However, the brain is lagging behind. Animal models show that CSF glucose concentration decreases at a lower rate than serum glucose concentration.[6] Due to the large number of intracellular osmolytes, the brain will be relatively hyperosmolar, leading to a water shift. In animal models of hyperglycaemia, treatment with insulin and hypotonic saline increases the amount of brain water by 8%.[5]

In conclusion, time is the crucial factor. Hyperglycaemia develops gradually. The brain adapts and the reduction of brain water will be minimal. Correction of hyperglycaemia usually occurs very quickly. When this is not opposed by an increase in serum sodium, a substantial increase of brain water will follow, eventually resulting in cerebral oedema.

Additional predisposing factors of cerebral oedema in DKA

Although the osmotic shifts during treatment appear to play a crucial role in the development of cerebral oedema, the exact underlying mechanism remains unclear.

An increasing amount of evidence suggests a multifactorial pathogenesis. In some patients with hyperglycaemia (of DKA), cerebral oedema is already present at admission, so factors unrelated to treatment are relevant as well.[7]

- *Vasogenic factors*
  DKA can lead to cerebral ischaemia. Severe volume depletion and hypocapnia (respiratory compensation for metabolic acidosis) may lead to vasoconstriction resulting in cerebral hypoperfusion and possibly cytotoxic oedema.[8,9] Volume resuscitation will lead to reperfusion of the ischaemic brain. Loss of autoregulation can cause hyperaemia and vasogenic oedema.[8-10]
- *Increased permeability of the blood-brain barrier*
  The permeability of the vascular endothelium increases due to a combination of hyperglycaemia (osmotic disruption), ketoacids, cerebral hypoxia and inflammation.[3] The blood-brain barrier will be less restrictive for insulin, sodium and water.[3,5] This will accelerate the development of cerebral oedema.
- *Acidaemia*
  Initially, there is an intracellular as well as an extracellular acidosis in DKA. Organic ketoacids acidify the cytosol, activating the Na+/H+ exchanger. Treatment with insulin will (relatively) alkalise the extracellular fluid and cause hyperstimulation of the Na+/H+ exchanger. The intracellular H+ ions will be exchanged for Na+, with intracellular accumulation of Na+ and H_2O and the development of oedema.[3] Animal studies support the role of insulin in the development of cerebral oedema. Both in rats[11] and rabbits[12] treatment of hyperglycaemia without insulin did not result in cerebral oedema. However, correction of hyperglycaemia with (high) dose insulin did lead to cerebral oedema. Especially early administration of insulin, when the blood-brain barrier is less restrictive for insulin, can cause a substantial effect.[7]
  Suppletion of bicarbonate during treatment of DKA will buffer the extracellular protons. This will stimulate the Na+/H+ exchanger even more and may therefore increase the formation of oedema.[3]

Treatment of DKA

Most knowledge regarding the development of cerebral oedema in DKA is based on small, retrospective paediatric studies. Cerebral oedema predominantly occurs in children and adolescents with an incidence 7 per 1000 episodes of DKA.[3] Cerebral oedema in adults with DKA is infrequently reported, although underreporting may be substantial.[13] Due to its infrequent occurrence, guidelines for the treatment of DKA in adults do not elaborate on the prevention of cerebral oedema. However, cerebral oedema is an insidious complication and when it does occur, its course can be disastrous with rapid clinical deterioration and a high mortality. Recently, a fatal case of cerebral oedema due to DKA in an adult patient was described in this journal.[14] Moreover, adolescents are regularly admitted to the ICU with severe DKA, due to irregular lifestyle and incompliance with therapy.

Furthermore, cerebral oedema may also present with subtle signs, such as memory disturbances, and may escape attention, therefore called 'subclinical'.[15] In more than half of the children with DKA subclinical oedema was present on MRI.[16] In adults this has not been properly investigated, although reversible subclinical cerebral dysfunction with sensory evoked potentials in the first hours of treatment has been demonstrated. [17]

Optimal rate of glucose and sodium correction and main parameter to guide therapy

The main parameter to guide therapy is effective serum osmolality. A fast decrease in serum glucose is acceptable as long as the effective serum osmolality remains more or less equal. As stated earlier, glucose correction in the brain lags behind as compared with the blood. When hyperglycaemia is corrected quickly with volume

resuscitation, insulin and restoration of diuresis, the brain will become relatively hyperosmolar. The sodium concentration will have to rise, otherwise cerebral oedema will develop.

Based on this pathophysiological view, the new Dutch paediatric guideline for DKA recommends to keep effective serum osmolality constant during the first 12-18 hours of treatment.[18] This advice is also based on a retrospective study which showed that in children who developed cerebral oedema, effective serum osmolality had decreased 9 mosmol/kg (or more) during the first four hours of treatment. In children without cerebral oedema, effective serum osmolality had hardly changed during the first hours of treatment.[19]

In the recently revised guideline for DKA in adults, a rather high correction rate of serum effective osmolality (<4 mosm/kg/h) is accepted. However, in the patient described in this journal, effective serum osmolality had decreased 22 mmol within five hours.[14] This is quite near the margins of the revised guideline. There is no proven benefit of very fast correction of the hypertonic state, therefore the guideline for adults could be more stringent regarding this issue.

How to avoid a fast decrease in effective serum osmolality? First, continuous low-dose administration of insulin is sufficient to stop production of ketoacids. There are no studies which demonstrate beneficial effects of administration of insulin as a bolus.[20] Normalisation of the serum glucose concentration has no priority. Potential dangers of a sudden high insulin level are a very quick decrease in glucose concentration, as well as stimulation of the Na+/H+ exchanger, both augmenting the risk of cerebral oedema.

Second, the infusion rate can be reduced. Although manifest haemodynamic instability rectifies the use of large volumes of infusate, it occurs infrequently in severe DKA. Therefore, frequently there is no need to use these large quantities of infusate.

Third, preferably isotonic fluids should be given. A rise in the serum sodium concentration is a sine qua non for maintaining a stable blood osmolality.

Physicians have been educated that increases of more than 8-10 mmol/24 hours are dangerous due to the risk of central pontine demyelination. However, this applies for *hypotonic* hyponatriaemia with a fast increase in effective serum osmolality. In DKA with hyperglycaemia and *hypertonic* hyponatraemia, the increase in sodium will only prevent a fast decrease of the effective serum osmolality. Therefore, this should not be a reason to switch to hypotonic fluids and a rise in serum sodium of more than 8 mmol/24 hours should be accepted.

CONCLUSIONS

Hyperglycaemia develops gradually with adaptation of the brain, and should be corrected slowly because the brain lags behind (natura non facit saltus). The main parameter to guide therapy is effective serum osmolality, which should be kept well within the margins defined in the revised Dutch guideline (<<4 mOsmol/kg/hour) but by preference remain equal during the first hours. Fast reduction of serum glucose is not dangerous as long as it is compensated by an opposite trend in serum sodium concentration. However, a bolus of insulin should be avoided since it may accelerate the development of cerebral oedema. Continuous insulin administration is sufficient to stop ketogenesis. The fast rise of serum sodium concentration during correction of hyperglycaemia (*hypertonic* hyponatriaemia) is a 'sine qua non' and not a reason to switch to hypotonic fluids, since (contrary to correction of *hypotonic* hyponatriaemia) effective serum osmolality does not increase.

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Case reports containing concise reports on original work will be considered for publication. Case reports which are relevant for understanding the pathophysiology or clinical presentation of disease may also be accepted under this heading. Selection of case reports will be based on criteria as outlined in a special report by the editors (Drenth et al. The case for case reports in *the Netherlands Journal of Medicine*. Neth J Med. 2006;64(7):262-4). We advise potential authors to take notice of the instructions in this report. Articles published in this section should be no longer than 1000 words, and supplied with a summary of about 60 words, preferably no more than two figures and/or tables, and no more than 15 references. In addition, we require that authors of case reports answer the following two questions (Neth J Med. 2008;66(7):289-90): 1) What was known on this topic? and 2) What does this add? The answers will appear in a separate box in the text.

Mini reviews
Mini reviews are concise notes that bring the reader up to date with the recent developments in the field under discussion. The review article should mention any previous important reviews in the field and contain a comprehensive discussion starting with the general background of the field. It should then go on to discuss the salient features of recent developments. The authors should avoid presenting material which has already been published in a previous review. The manuscript should be divided as follows: title page, abstract and main text. The text may be subdivided further according to the areas to be discussed. The text should not exceed 2500 words.

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Letters to the editor will be considered by the editorial board. Letters should be no more than 400 words. Please use SI units for measurements and provide the references conform the Vancouver style (N Engl J Med. 1991;324:424-8). No more than one figure is allowed. For letters referring to articles previously published in the Journal, the referred article should be quoted in the list of references.

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A photo quiz should not exceed 500 words and include no more than two figures and four references conform the Vancouver style. Abbreviations of measurements should be quoted in SI units.

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