PHOTO QUIZ: Blurred vision, see page 461

Hereditary haemochromatosis

Probiotics and ulcerative colitis

Incidence of first acute myocardial infarction
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The mission of the journal is to serve the need of the internist to practise up-to-date medicine and to keep track with important issues in health care. With this purpose we publish editorials, original articles, reviews, controversies, consensus reports, papers on specialty training and medical education, book reviews and correspondence.

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DECEMBER 2007, VOL. 65, NO. 11
Hereditary haemochromatosis

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In the current issue of the Netherlands Journal of Medicine, some new developments in the field of hereditary haemochromatosis (HH) are discussed. Swinkels et al. publish the recently developed guidelines for diagnosis and management of HH on behalf of the Dutch Institute for Healthcare Improvement, the CBO.1 Jacobs et al. review changing aspects of HFE-related HH2 and report the results of family screening.3 HH is one of the most common inherited disorders with an autosomal recessive inheritance pattern. Initial clinical symptoms are relatively aspecific, making it difficult to recognise them as related to iron overload. In later stages, disease manifestations may include arthropathy, diabetes mellitus, hypogonadism and other endocrinopathies, liver cirrhosis, cardiomyopathy, skin pigmentation, and in cirrhotic patients, increased susceptibility to liver cancer. Early diagnosis and therapeutic phlebotomy can prevent the development of tissue damage, reducing morbidity and mortality, and providing long-term survival similar to the general population. Unfortunately, vague symptoms such as arthropathy and tiredness often persist after therapy.

In 1996, Feder et al. identified the haemochromatosis (HFE) gene (previously called HLA-H gene). They attributed the most common form of HH to homozygosity for the C282Y sequence variation of this gene. Since then, it rapidly became clear that the situation was much different than previously thought: despite its remarkably high prevalence C282Y homozygosity was characterised by relatively low penetrance. Recent surveys involving HFE genotyping of nonclinically selected populations found that a large number of C282Y homozygotes had no symptoms of disease. Heterogeneity of clinical presentation, even within families, is reported, suggesting that there is a role for other unknown genetic and environmental factors.

HFE genotypes other than C282Y homozygosity rarely cause clinically significant iron overload. C282Y heterozygotes usually do not develop iron overload unless they have associated conditions, such as environmental factors (alcohol, viruses, hepatic disease) or variant forms of other genes. A particular group of HFE genotypes consists of persons who are compound heterozygous for C282Y and H63D. These individuals have been described as being at higher risk to develop iron overload, but generally in a much milder form than in C282Y homozygotes. However, given the fact that the clinical penetrance of C282Y homozygosity is low, compound heterozygotes with clinical disease will be scarce. A third sequence variant, S65C, with an allele frequency as low as 1.6 to 2.0%, was found to exert a consistent but small effect on serum iron indices, particularly when present in combination with other HFE genotypes, such as C282Y and H63D.

The molecular function of HFE in iron metabolism has long been attributed to the crypt hypothesis. However, it is mainly since the discovery of hepcidin that the crypt model has been replaced by the hepcidin model as the prevailing hypothesis. The recently identified β-defensin-like antimicrobial peptide hepcidin is thought to be the long-anticipated regulator that controls iron absorption and macrophage iron release. Hepcidin is synthesised in the liver when changes occur in body iron needs, such as in anaemia, hypoxia and inflammation, and is secreted in the circulation. Recently, light was also shed on how hepcidin exerts this regulatory function; it was reported to counteract the function of ferroportin, a major cellular iron-exporter protein in the membranes of macrophages and the basolateral site of enterocytes, by inducing its internalisation and degradation. Sequence variations in HFE were shown to lead to inappropriately low concentrations of hepcidin, suggesting that HFE is involved upstream in the regulation of hepcidin expression. In the future, determination of hepcidin might be a valuable tool in the diagnosis of atypical cases of anaemia and haemochromatosis.

According to the guideline, elevated serum ferritin in combination with transferrin saturation (TS) above 45% is suggestive of the presence of primary iron overload. Discussion is going on about the exact reference values, due to the different populations examined and the variability of normal ferritin values between laboratories. Unfortunately,
an increasing number of patients undergo molecular testing just because plasma ferritin or TS is increased. Often this leads to an unnecessary search for hereditary defects in individuals with various common, non-hereditary conditions that are characterised by similar abnormalities in serum ferritin and/or TS, such as hepatitis, excessive alcohol consumption and secondary forms of iron overload. There is increasing evidence concerning the relation between elevated serum ferritin levels and the metabolic syndrome, but the pathophysiology and clinical consequences are not clear yet. In these cases TS is generally normal.

The gold standard for diagnosis of liver iron overload remains a liver biopsy. According to the guideline (which is mainly expert-opinion based) a liver biopsy is indicated in the following cases: 1) elevated liver enzymes in combination with HH and 2) serum ferritin above 1000 µg/l. A relatively new diagnostic tool for the presence and severity of iron overload is magnetic resonance imaging (MRI). In case of elevated ferritin levels in the absence of homozygosity for C282Y / compound heterozygosity for C282Y/H63Asp hepatic iron quantification with MRI might be helpful. However, consensus has not been reached yet regarding the technique or the possibility to reproduce the same method of calculus in different machines. Of course, the advantage of a biopsy is that histology may show cirrhosis and fibrosis, which may change the prognosis of the patient.

Treatment of HH is relatively simple, reducing iron accumulation by phlebotomy. With removal of 500 ml of blood, 200 to 250 mg iron is removed from the body. Treatment starts with intensive phlebotomy, weekly phlebotomy until a serum ferritin level of 50 µg/l is reached. Thereafter it is not clear whether one should hold on to a ferritin level of 50 µg/l or a higher level. Red cell apheresis is considered to be an alternative procedure; it is suggested that it removes excess iron twice as fast as manual whole blood phlebotomy. Currently this method is being evaluated as treatment of HH in the Netherlands.

It is suggested that the majority of relatives found to be homozygous for the C282Y mutation will have biochemical evidence of iron overload and 10 to 38% may have HH-associated liver disease or arthropathy. Siblings of a subject homozygous for the C282Y mutation have a one in four chance of inheriting the same mutation if both parents are heterozygous, or a one in two chance if one parent is homozygous and one is heterozygous. Therefore, family screening has been proposed, since this has proven efficacy in the detection of latent homozygotes for frequent recessive mutations. In the Hemochromatosis Family Study (HEFAS) study Jacobs et al. describe that morbidity among first-degree family members of C282Y-homozygous probands previously diagnosed with clinically proven HH is higher than that in an age- and gender-matched normal population.³ For clinicians, the challenge is now to diagnose HFE-related HH before irreversible tissue damage appears and at the same time to distinguish HH from increasingly common diseases that lead to only moderately increased body iron stores, such as the metabolic syndrome. The other challenge is to optimally use both conventional and innovative laboratory tests to differentiate between the various causes of iron overload. After initial clinical and laboratory investigations and exclusion of acquired causes of hyperferritinaemia, atypical patients should be referred to specialised centres that can perform investigations with an up-to-date, targeted approach. However, the strategy proposed may change in time with advances in noninvasive techniques for the assessment of hepatic iron and tissue damage, the availability of hepcidin measurements in both urine and serum, and the identification of new key players in iron homeostasis.

REFERENCES

Probiotics and remission of ulcerative colitis: a systematic review

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ABSTRACT

Background: Ulcerative colitis (UC) is an acute and inflammatory disease of the large bowel of unknown aetiology. The use of probiotics for this disease remains controversial. The objective of this systematic review was to identify studies based on randomised controlled trials comparing the effect of probiotics to the effect of anti-inflammatory drugs or placebo in the remission of UC.

Methods: We conducted a systematic review of clinical trials comparing the effect of probiotics to the effect of anti-inflammatory treatment or placebo in the remission of UC. PubMed, ScienceDirect, Cochrane, Google Scholar, metaRegister of Controlled Trials and National Institutes of Health were searched.

Results: Nine studies met the inclusion criteria. These studies present a significant heterogeneity concerning their methodology and their results. The improvement in UC remission and the frequency of adverse effects do not differ significantly between probiotic and control groups.

Conclusions: There are a limited number of randomised trials published in the field of probiotics used for the remission of UC, and they present many methodological differences. The existing studies suggest a similar safety and efficacy of probiotics in comparison with anti-inflammatory drugs.

KEYWORDS

Clinical trials, probiotics, ulcerative colitis, randomised

INTRODUCTION

Ulcerative colitis (UC) is a relapsing disease of the colon of unknown aetiology. Clinical studies and experiments in animals suggest that genetic factors, agents such as viruses or other micro-organisms, reactions to allergens (milk proteins and bacterial polysaccharides), autoimmune phenomena or a combination of these may have a role in the aetiology of this condition. Its annual incidence is about 10 new cases per 100,000 white adults at risk.¹ An attractive therapy for UC manipulation is to reduce the inflammatory effectiveness of colonising bacteria. Antibiotics are one option to eliminate the species involved in inducing the inflammation.² Antibiotics are generally not effective for acute UC.¹ In spite of this, aminosalicylates are recommended for maintenance treatment.³ However, there is considerable intolerance not only to classic aminosalicylate sulphalazine⁴ but also to sulphur-free compounds such as mesalazine or olsalazine.⁵ Current 5-aminosalicylate formulations have positive results in the majority of patients but they are associated with a number of limitations such as inconvenient dosing regimens and poor patient acceptance leading to noncompliance with prescribed therapy.⁶ An alternative is to use probiotic bacteria that interact with the host epithelium to resolve inflammation. Probiotics have been defined as live microbial feed supplements that beneficially affect the host by improving the intestinal microbial balance. Theoretically, probiotics can modify the composition and some metabolic activities of microflora by preventing overgrowth of potentially pathogenic bacteria.⁷,⁸ The relationship between intestinal inflammation and pathogenic bacteria is perplexing. Similarly, the field of probiotics is complex and in need of rigorous research.⁹,¹⁰ If bacteria participate in the pathogenesis of ulcerative colitis and in resistance to antibiotics, probiotics may offer an alternative useful way to manipulate the microflora in chronic diseases.¹⁰ Several studies suggest that selected probiotic preparations have a positive influence...
in gastrointestinal diseases including UC.\textsuperscript{11-13} The most widely used probiotics in humans are \textit{Bifidobacteria} and \textit{Lactobacilli}. However, data are based on relatively small studies, which are not sufficient to determine if they are definitely helpful, and the benefits and harms implicated are still poorly understood.\textsuperscript{14}

The objective of this systematic review was to identify studies based on data of randomised controlled trials comparing the effect of probiotics with the effect of anti-inflammatory drugs or placebo in the remission of UC in order to compare their methodology and summarise their results.

\section*{Materials and Methods}

\subsection*{Criteria for study selection}

Abstracts and full articles of all citations and retrieved studies comparing the effects of probiotics with those of anti-inflammatory drugs or placebo, published before 9 October 2007 were reviewed and rated for inclusion. Full articles were retrieved if specific treatments were given to treat the disease of interest. The inclusion criteria were randomised, controlled trials in humans addressing probiotic use for the induction of remission and/or maintenance of remission. Exclusion criteria were preclinical studies, case reports or case series, phase 1 studies in volunteers and not in the disease being studied.

\subsection*{Data sources and data extraction}

The databases searched for unrestricted dates and languages until 9 October 2007 were PubMed, ScienceDirect, Cochrane and Google Scholar. Two on-line clinical trial registers were searched: metaRegister of Controlled Trials (www.controlled-trials.com/mrct), and National Institutes of Health (www.clinicaltrials.gov). A secondary hand search of reference lists, authors, associated diseases and meeting abstracts was also performed. The key words used to search in PubMed were (lactobacillus OR probiotics OR saccharomyces OR bifidobacterium OR yeasts OR yogurt OR dairy products) AND ulcerative colitis. In ScienceDirect and Google Scholar we used probiotics and ulcerative colitis and in Cochrane, metaRegister of Controlled Trials and National Institutes of Health the keyword was probiotics. Search strategies were broad-based initially, and then narrowed to the disease of interest.

Data on general characteristics of patients, patients at the start of the study, number of completed subjects, treatment type and duration, outcomes and adverse effects were extracted into a standardised table. One researcher completed the search and checked all titles and abstracts of relevant studies. Two authors reviewed the full text of relevant studies for their eligibility for inclusion. When discrepancies occurred a third author resolved them. Two trials had multiple arms.\textsuperscript{15,16} In one trial the two groups of patients receiving anti-inflammatory drugs were considered as one control group.\textsuperscript{15} The second trial included two probiotic groups.\textsuperscript{16} Each one of them was compared with the control group separately.

\subsection*{Methodological quality}

Each study included in the systematic review was evaluated on the following items: inclusion and exclusion criteria for patients, co-treatment/concomitant medication use, and outcome measurement. For inclusion/exclusion criteria we examined if inclusion and exclusion criteria are clearly stated in the text. For co-treatment we examined if concomitant medication was used in the probiotic group. For the outcome measurement we examined if a clinical activity index and/or an endoscopy index were used at entry and at the end of the study for each patient.

\subsection*{Statistical analysis}

Summary statistics were performed using the software Lau-Meta-analyst.EXE. Relative risks with 95\% confidence intervals were computed as summary statistics. Heterogeneity across trials was evaluated using Cochran’s Q test. Regardless of whether the studies were homogeneous or not, a random effects model was used and a pooled relative risk was calculated using the DerSimonian and Laird method. P values <0.05 were considered statistically significant.

\section*{Results}

\subsection*{Results of searching}

A total of 24 articles were initially identified, comparing the effect of probiotics with the effect of anti-inflammatory drugs or placebo (table 1). The other papers contained general information about probiotics and inflammatory bowel disease. All these papers were found in PubMed using the key words mentioned above. As shown in table 1, 15 articles failed to meet one or more of the inclusion criteria. Five studies were not randomised controlled trials,\textsuperscript{19,21-23,30,35-36} four referred to pouchitis,\textsuperscript{19,25-27,31,34} one referred to inflammatory bowel disease,\textsuperscript{13} one to colonic surgery,\textsuperscript{24} three to Crohn’s disease\textsuperscript{41-43,36} and one\textsuperscript{47} was published twice. Nine studies met the inclusion criteria and provided data on 972 enrolled subjects. The number of patients in each of these studies ranged from 18 to 327 (median 103). The included studies are presented in table 2. One study used a symbiotic compared with placebo in patients with active UC.\textsuperscript{22} One study used balsalazide and VSL\#3 compared with mesalazine and balsalazide in patients with mild to moderate UC.\textsuperscript{15} One study used \textit{Lactobacillus GG} compared with mesalazine and with \textit{Lactobacillus GG} plus mesalazine.\textsuperscript{46} Three studies used \textit{E. coli} compared with mesalazine in active and in inactive UC\textsuperscript{48-50} and

\textsuperscript{412}
three studies used *Bifidobacteria* compared with placebo in mild to moderate and in active UC. Concerning the methodological quality, the studies present significant differences, and only four of them combine clear inclusion and exclusion criteria, exclusive use of probiotics in the experiment group, and adequate outcome measurement (table 3).

**Clinical success of experiment-control group**

Among nine randomised, controlled studies providing adequate data, two reported a significantly higher remission in UC for the probiotics compared with the control group. Two studies showed a trend for increased efficacy and five trials did not show any significant difference between probiotic and control groups. The pooled relative risk for the nine randomised-controlled trials was 1.51 (95% CI 0.79-2.87, p=0.21) (table 4), showing no statistically significant difference between probiotic and control groups. A nonsignificant heterogeneity was found (Q=5.47). The normal heterogeneity for 6 df according to the χ² distribution is 10.645.

**Subgroups of studies**

*Induction of remission vs maintenance of remission*

Three randomised, controlled studies estimated induction of remission as an outcome measure. One of them reported significantly improved remission in UC for the probiotics compared with the control group. The other two studies had a trend for increased efficacy. The pooled relative risk was 2.27 (95% CI 1.00-5.14, p=0.049), showing a significant difference between probiotic and control group. A nonsignificant heterogeneity was found (Q=0.20) as the normal heterogeneity for 2 df according to the χ² distribution was 4605.

Six randomised, controlled studies provided adequate data for the maintenance of remission. Two of them reported significantly higher remission in UC for the probiotics compared with the control group. The other four trials did not find any significant difference between the probiotic and control group. The pooled relative risk was 1.37 (95% CI 0.62-3.04, p=0.44) showing no significant difference between probiotic and control group. A nonsignificant heterogeneity was found (Q=24.26) as the normal heterogeneity for 6 df according to the χ² distribution was 10.645.

---

**Table 1. Studies on probiotics and inflammatory bowel disease**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Disease</th>
<th>Randomised controlled trial</th>
<th>Probiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tursi et al.</td>
<td>2004</td>
<td>Ulcerative colitis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Zocco et al.</td>
<td>2006</td>
<td>Ulcerative colitis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ishikawa et al.</td>
<td>2002</td>
<td>Ulcerative colitis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Kruis et al.</td>
<td>2004</td>
<td>Ulcerative colitis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Braegger et al.</td>
<td>2003</td>
<td>Pouchitis</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Rembacken et al.</td>
<td>1999</td>
<td>Ulcerative colitis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Bibiloni et al.</td>
<td>2005</td>
<td>Ulcerative colitis</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Furrie et al.</td>
<td>2005</td>
<td>Ulcerative colitis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Annese et al.</td>
<td>2004</td>
<td>Inflammatory bowel disease</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Kato et al.</td>
<td>2004</td>
<td>Ulcerative colitis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Schultz et al.</td>
<td>2004</td>
<td>Crohn's disease</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Teml et al.</td>
<td>2003</td>
<td>Crohn's disease</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cui et al.</td>
<td>2003</td>
<td>Ulcerative colitis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Gionchetti et al.</td>
<td>2000</td>
<td>Pouchitis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Folwaczyn</td>
<td>2000</td>
<td>Ulcerative colitis</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Faubion et al.</td>
<td>2000</td>
<td>Ulcerative colitis</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Kruis et al.</td>
<td>1997</td>
<td>Ulcerative colitis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Everett et al.</td>
<td>1969</td>
<td>Colonic surgery</td>
<td>Maybe</td>
<td>No</td>
</tr>
<tr>
<td>Kuhbacher et al.</td>
<td>2006</td>
<td>Pouchitis</td>
<td>Maybe</td>
<td>Yes</td>
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<tr>
<td>Gionchetti et al.</td>
<td>2003</td>
<td>Pouchitis</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Cui et al.</td>
<td>2004</td>
<td>Ulcerative colitis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Bai et al.</td>
<td>2006</td>
<td>Ulcerative colitis</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Shibata et al.</td>
<td>2007</td>
<td>Ulcerative colitis</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Van Gossum et al.</td>
<td>2007</td>
<td>Crohn's disease</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Table 2. Characteristics of nine randomised controlled trials assessing the effect of probiotics in ulcerative colitis remission

<table>
<thead>
<tr>
<th>Authors, year</th>
<th>Probiotic</th>
<th>Control group</th>
<th>Dose (n of probiotic/ day)</th>
<th>Treatment duration</th>
<th>N (probiotic/control group)</th>
<th>Disease severity</th>
<th>Induction or maintenance of remission N (probiotic/control group)</th>
<th>Outcome measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tursi et al. 2004</td>
<td>Balsalazide/ VSL#3</td>
<td>Mesalazine/ balsalazide</td>
<td>$900 \times 10^8$</td>
<td>8 weeks</td>
<td>30/30/30</td>
<td>Mild-to-moderate</td>
<td>Induction of remission N (24/21/16)</td>
<td>1. Patients in symptomatic remission based on clinical evaluation and diary card 2. Time to symptomatic remission, proportion of patients with improvement in endoscopic and histological score</td>
</tr>
<tr>
<td>Zocco et al. 2006</td>
<td>Lactobacillus GG</td>
<td>Mesalazine</td>
<td>$18 \times 10^9$</td>
<td>12 months</td>
<td>65/60/62</td>
<td>Inactive UC</td>
<td>Maintenance of remission N (55/48/52)</td>
<td>1. Number of patients suffering relapse among the 3 groups 2. To evaluate the variations of clinical, endoscopic and histological scores and the relapse-free time as index of drug efficacy</td>
</tr>
<tr>
<td>Ishikawa et al. 2002</td>
<td>Bifidobacterium breve Bifidobacterium bifidum Lactobacillus acidophilus YIT 0168</td>
<td>BFM without these Bifidobacteria</td>
<td>$10 \times 10^8$</td>
<td>12 months</td>
<td>11/10</td>
<td>Mild Moderate</td>
<td>Maintenance of remission N (7/1)</td>
<td>Exacerbation of clinical symptoms</td>
</tr>
<tr>
<td>Kruis et al. 2004</td>
<td>E. coli Nissle 1917</td>
<td>Mesalazine</td>
<td>$2.5-25 \times 10^9$</td>
<td>12 months</td>
<td>162/165</td>
<td>Inactive</td>
<td>Maintenance of remission N (89/104)</td>
<td>Comparison of number of patients with relapse of UC between the two groups</td>
</tr>
<tr>
<td>Rembacken et al. 1999</td>
<td>E. coli Nissle 1917 serotype O6: K5: H1</td>
<td>Mesalazine</td>
<td>$5 \times 10^{10}$</td>
<td>12 months</td>
<td>57/59</td>
<td>Active</td>
<td>Maintenance of remission N (39/44)</td>
<td>1. Time and rate of relapse 2. Time and rate of remission in patients treated with topical or systemic steroids in addition to the non-pathogenic E. coli or mesalazine</td>
</tr>
<tr>
<td>Furrie et al. 2005</td>
<td>Synbiotic (Bifidobacterium longum + inulin-oligofructose)</td>
<td>Potato starch and sachet of 6 g powdered maltodextrose</td>
<td>$4 \times 10^{10}$</td>
<td>4 weeks</td>
<td>9/9</td>
<td>Active</td>
<td>Induction of remission N (5/3)</td>
<td>1. Clinical improvement in symbiotic vs placebo group 2. Effects of symbiotic in mucosa</td>
</tr>
<tr>
<td>Kato et al. 2004</td>
<td>Bifidobacterium breve strain Yakult Bifidobacterium bifidum strain Yakult Lactobacillus acidophilus</td>
<td>BFM without B. bifidum and L. acidophilus</td>
<td>$10^9$</td>
<td>12 weeks</td>
<td>10/10</td>
<td>Mild-to-moderate active</td>
<td>Induction of remission N (4/3)</td>
<td>Clinical improvement (indicated by a decrease in CAI score of ≥3 points)</td>
</tr>
<tr>
<td>Cui et al. 2004</td>
<td>Bifidobacteria</td>
<td>Starch</td>
<td>$1.26 \text{ g/d}$</td>
<td>8 weeks</td>
<td>15/15</td>
<td>Active</td>
<td>Maintenance of remission N (12/1)</td>
<td>Effects of probiotics on intestinal mucosa and role of probiotics in preventing relapse of UC</td>
</tr>
<tr>
<td>Kruis et al. 1997</td>
<td>E. coli Nissle 1917 serotype O6: K5: H1</td>
<td>Mesalazine</td>
<td>$50 \times 10^5$</td>
<td>12 weeks</td>
<td>50/53</td>
<td>Inactive UC</td>
<td>Maintenance of remission N (42/51)</td>
<td>Prove equivalence of the CAI score under the E. coli and mesalazine</td>
</tr>
</tbody>
</table>

N = number of patients.
Probiotics vs anti-inflammatory drugs and vs placebo

Trials that compared the effects of probiotics with the effect of placebo (Bifidobacteria vs placebo, synbiotic vs placebo) gave better results than studies that compared the effect of probiotics with the effect of anti-inflammatory drugs. Among five randomised, controlled studies comparing probiotics with anti-inflammatory drugs, Tursi's trial showed a trend for increased efficacy. The other four studies did not find any significant difference between probiotics and anti-inflammatory agents. The pooled relative risk was 0.95 (95% CI 0.98-1.55, p=0.84), showing no significant difference between probiotic and anti-inflammatory treatment. A nonsignificant heterogeneity was found (Q=9.63) as the normal heterogeneity for 5 df according to the $ \chi^2 $ distribution was 9.236.

Among four randomised, controlled studies with probiotics with placebo, two trials reported significantly higher remission in UC for patients receiving probiotics. The other two trials showed a trend for increased efficacy of probiotic compared with placebo. The pooled relative risk was 7.32 (95% CI 1.57-39.13, p=0.020), showing a significant difference between probiotic and placebo. A significant heterogeneity was found (Q=7.42).

**Type of probiotic and ulcerative colitis**

Significant differences in effectiveness have also been reported for different types of strains in species of bacteria and yeasts. Depending on the type of probiotic, the clinical success of the Bifidobacteria treatment combined with one synbiotic was significantly more effective compared with...
the control group in contrast to the studies with *E. coli*, which did not present significantly improved effect for the probiotic group: *Bifidobacteria* vs control group: odds ratio 7.32 (1.37–39.13), *E. coli* vs control group: odds ratio 0.66 (0.43–1.02). The type of UC does not seem to influence the results: mild-to-moderate UC: odds ratio 3.39 (0.97–11.87), active UC: odds ratio 1.79 (0.37–39.01), nonactive UC: odds ratio 1.26 (0.64–2.46) (table 4).

### Adverse effects into subgroups of studies

In all subgroups mentioned above the frequency of adverse effects did not differ significantly between the probiotic and the control group. The pooled relative risks of adverse effects for each subgroup were: probiotics vs anti-inflammatory drugs: 1.12 (0.69–1.83), probiotics vs placebo: 0.72 (0.10–5.30), induction of remission: 0.29 (0.06–1.45), maintenance of remission: 1.27 (0.86–1.86). The pooled relative risks of adverse effects for the different species of probiotics and types of UC were: *Bifidobacteria*: 0.72 (0.10–5.30), *E. coli*: 1.25 (0.85–1.84). For different types of UC the pooled relative risks for adverse effects were: active UC: 0.83 (0.12–5.94), nonactive UC: 1.16 (0.77–1.74), mild to moderate UC: 0.60 (0.12–3.08).

### Discussion

According to the results of this systematic review, there are only few randomised trials assessing the effectiveness and safety of probiotics used for the remission of UC. These studies suggest that probiotics do not differ significantly from anti-inflammatory drugs for UC remission, concerning both effectiveness and safety. A significant heterogeneity of results was found among studies. The contradictory results of randomised trials may arise from methodological differences between studies, such as the type of probiotic being investigated, or differences in duration of treatment.

Significant differences in effectiveness have been reported for different types of strains in species of bacteria and yeasts. For UC, additional factors may influence the results, including the type of UC, medication compliance and patient behaviour. Another source of heterogeneity for probiotic trials is the use of antibiotics together with probiotics, the differences in control groups, the outcome measures, and the number of patients included in each study.

According to the results of the present study *Bifidobacteria* are likely to give the best results. The efficacy of the *Bifidobacteria* may be related to the increased concentrations of faecal (luminal) short chain fatty acids (SCFAs), and these probiotics may improve epithelial function via production of SCFAs. SCFAs, particularly butyrate, are the major energy source for colonocytes and appear to function in immunological regulation including the suppression of proinflammatory cytokines through the inhibition of NF-κB activation. *Bifidobacteria*–femented milk (BFM) supplements may also reduce exacerbation of UC through the normalisation of the intestinal flora and may lead to a significant decrease in the relative number of *B. vulgatus* (percentage) in *Bacteroidaceae* in faeces. However, another explanation for the improved results of *Bifidobacteria* could be that all studies using *Bifidobacteria* as a probiotic used placebo (and no anti-inflammatory drugs) for the control group. In addition, these studies are based on small numbers of patients.

The results of our study suggest no significant difference in effectiveness between *E. coli* and anti-inflammatory drugs. Several factors may be related to this finding. A recent controlled trial suggests an effectiveness of ciprofloxacin in complicated UC. Oral tobramycin was shown to eliminate pathogenic *E. coli* strains; this was related to significant clinical and histological improvement of UC. However, when tobramycin was stopped, pathogenic adhesive *E. coli* recoloured, and relapses occurred in some patients. We hypothesise that this may also happen with other drugs, such as mesalazine, giving another possible explanation for the results of these trials. It should be pointed out that all three trials for *E. coli* included in the systematic review compared the probiotic group with a control group receiving mesalazine and not placebo, while trials for *Bifidobacteria* used placebo in the control group. As a consequence, it is difficult to conclude that *E. coli* is less effective than *Bifidobacteria* in UC remission.

Trials using probiotics vs placebo are likely to give better results than trials using probiotics vs antibiotics. The difference may be related to the fact that all the trials comparing probiotics with placebo used *Bifidobacteria*, as a probiotic, with clearly better results in effectiveness than other probiotics mentioned above. The trials comparing probiotics with anti-inflammatory drugs, use *E. coli* or VSL#3 or *Lactobacillus* as a probiotic, and did not show a significant difference in effectiveness between probiotic and control groups. However, this finding may be related to a similar effectiveness of probiotics and anti-inflammatory drugs, and not to a lower effectiveness of the specific probiotics used in these trials.

The present study found that trials assessing induction of remission as an outcome measure give better results for patients receiving probiotics than the trials assessing maintenance of remission. Why this occurred is not clearly understood. We hypothesise that the type of probiotic (most of the trials assessing induction of remission as outcome measure used *Bifidobacteria*) may be related to this finding.

Another limitation in the interpretation of our results could be related to the antibiotics the patients took before...
entering the study. The trials that had patients taking antibiotics before entering the study (three studies using Bifidobacteria as a control group) showed better results than the trials with patients who did not use antibiotics. The explanation of this finding is not clear. The type of UC, the antibiotic, the dose of the antibiotic and other factors must be taken into consideration.

Concerning the adverse effects, they do not present significant differences between probiotics and the placebo or pharmaceutical treatment. The results of adverse effects did not present significant heterogeneity among studies. The type of probiotic, the type of UC, or other methodological differences of the studies are not likely to influence the adverse effects to a significant level. Concerns about the safety of probiotics have been raised. As probiotics are living organisms given to ill patients, the threat for adverse reactions exists. Some intestinal bacteria have been shown to translocate from the intestine to other organs and antibiotic-resistance gene acquisition is also a concern. Considering that, globally, millions of doses of probiotics are taken a year, the risk of adverse effects due to probiotics is extremely low. Compared with many pharmaceutical agents, serious adverse effects from probiotics rarely occur because they are well tolerated and safe. While most of the species and genera, especially Lactobacilli and Bifidobacteria are apparently safe, certain micro-organisms may be problematic, particularly the Enterococci, which are associated with nosocomial infections and harbour transmissible antibiotic resistance determinants. However, prolonged safety issues have not been addressed in studies.

Positive results from the use of probiotics have been suggested by meta-analysis published by McFarland on travellers diarrhoea, Souza et al. on antibiotic associated diarrhoea, van Niel et al. on acute infectious diarrhoea in children. Sazawal et al. on acute diarrhoea and McFarland on antibiotic-associated diarrhoea. There are also positive results in meta-analysis published by Huang et al. on acute diarrhoea in children and Cremonini et al. on antibiotic-associated diarrhoea. A meta-analysis by Szajewska and Mrukowicz found moderately effective results for Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea. A micro-organism classified as a probiotic has to have non-pathogenic characteristics, be viable in delivery vehicles, be stable in acid and bile, adhere to target epithelial tissue, persist within the gastrointestinal tract, produce antimicrobial substances, modulate the immune system and influence metabolic activities. The variety of micro-organisms that have these requirements may or may not have similar impacts on specific health outcomes. The main advantage of probiotic therapies is that they are therapeutically active but they do not disrupt the re-establishment of the protective normal microbial flora. The way in which probiotics affect the gut is of much interest. To overcome the problems of gastrointestinal infection, a probiotic must be non-pathogenic and must act against pathogens in ways different than antibiotics, for example, by competition. Moreover, probiotics should have a rapid onset of action and survive the challenges of gastric acid, bile, or concurrent antibiotics. It is also important that they modify immune processes to help destroy the invading organism. The results of the present review suggest that probiotics, in general, are not more safe and effective than anti-inflammatory drugs in the remission of UC. But according to the type of probiotic or the type of UC they may be effective in the remission of UC. However, the systematic review showed that the number of studies published on this field is limited, with many methodological differences and a significant heterogeneity of results.

In conclusion, we can say that whether the use of probiotics can actually reduce the relapse of UC, and whether they are safer and more effective than anti-inflammatory drugs are issues that need to be further studied in clinical trials. The bacteria chosen, the dose of bacteria, and the duration of therapy all require further clarification. Continued investigation into the ways by which appropriate bacteria may prevent or ameliorate the chronic inflammatory state is necessary.

**REFERENCES**

Changing aspects of \textit{HFE}-related hereditary haemochromatosis and endeavours to early diagnosis


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\section*{ABSTRACT}
\textit{HFE}-related hereditary haemochromatosis (HH) is an iron overload disease attributed to the highly prevalent homozygosity for the C282Y mutation in the \textit{HFE} gene. The pathophysiology of this error in iron metabolism is not completely elucidated yet, although deficiency of the iron regulatory hormone hepcidin appears to play a role. Ways of diagnosing iron overload include measurement of the serum iron parameters, i.e. serum transferrin saturation and serum ferritin, by a liver biopsy or by calculating the amount of mobilisable body iron withdrawn by phlebotomies. Clinical signs attributed to \textit{HFE}-related HH include liver failure, arthralgia, chronic fatigue, diabetes mellitus and congestive heart failure. Organ failure can be prevented by phlebotomies starting before irreversible damage has occurred. Therefore, screening to facilitate early diagnosis is desirable in individuals at risk of developing \textit{HFE}-related iron overload. Over time it appeared that the clinical penetrance of the \textit{HFE} mutations was much lower than had previously been thought. This changed the opinion about a suitable screening modality from case detection, via population screening, to family screening as the most appropriate method to prevent \textit{HFE}-related disease. However, before the implementation of family screening it is vital to have thorough information on the relevance of the specific health problem involved, on the clinical penetrance of C282Y homozygosity and on the effectiveness of the screening approach.

\section*{KEYWORDS}
Diagnosis, family, hereditary haemochromatosis, \textit{HFE}, screening

\section*{INTRODUCTION}
Classical hereditary haemochromatosis (HH) is a disease related to iron overload with an increase in physical symptoms over time, leading to organ failure and poor survival. Treatment is relatively simple: removing iron overload by phlebotomies, thereby preventing disease and increasing survival. After the discovery of its prime gene mutation, the C282Y mutation of the \textit{HFE} gene, large-scale screening for \textit{HFE}-related HH became feasible. However, along the years it became clear that the traditionally low prevalence of patients with HH could not be fully ascribed to the ignorance of the medical staff, but was likely to be due to the limited penetrance of the \textit{HFE} gene mutation. This review describes new insights into pathophysiology, diagnosis and penetrance of \textit{HFE}-related HH, and its implications for secondary prevention and early treatment of the clinical disease.

\section*{HISTORY}
One of the first to describe a clinical syndrome characterised by cirrhosis of the liver, diabetes mellitus
and bronze skin pigmentation was Trousseau. The name haemochromatosis was first used by von Recklinghausen (1889), describing post-mortem findings in patients who had died from ‘bronzed diabetes’. In 1935, Sheldon suggested a familial form of haemochromatosis, but it was not until 1975 that Simon et al. described an autosomal recessive form of idiopathic haemochromatosis related to the HLA-A3 allele in the major histocompatibility complex (MHC) on chromosome 6. In 1996 Feder et al. were able to isolate the HH gene in 85% of HH patients. It was initially called HLA-H, as its organisation and structure were similar to genes in the HLA region that coded for HLA-class I heavy chains. However, as a HLA-class I pseudo gene had already been named HLA-H, the newly identified haemochromatosis gene was renamed HFE (the abbreviation of HFE being surprisingly not otherwise specified) as proposed by the Genome Databank.

Until now, more than 30 allelic variants of the HFE gene have been reported. The most common mutation is C282Y that results from a transition at nucleotide 845 (845G→A), leading to substitution of tyrosine for cysteine. This alters the HFE protein and its association with β2-microglobulin, resulting in a decreased presentation of the HFE protein on the cell surface. A second, although less important, HH-associated mutation occurs at nucleotide 187 of the HFE gene, with a substitution of histidine for aspartate at nucleotide 63 (63H→D). Several other HFE mutations, some of unknown significance, have been reported.

**PREVALENCE OF C282Y HFE GENE MUTATION**

The prevalence of the C282Y HFE gene mutation varies throughout the world. The overall prevalence of homozygosity and heterozygosity for the C282Y mutation in European countries is 0.4 and 9.2%, respectively, with heterozygosity ranging from 1% in the Southern European countries to 24.8% in Ireland. In North America an overall frequency of C282Y heterozygosity, regardless of the ethnic roots, was reported as 9.0%, whereas in the Indian subcontinent, and African, Middle Eastern and Australian populations prevalences of 0 to 0.5% were found. For the Netherlands the percentages of C282Y homozygosity and heterozygosity are calculated at 0.2 and 12.0%, respectively.

**PATHOPHYSIOLOGY**

The exact role of the mutated HFE in the pathophysiology of iron overload is still unclear. It has been suggested that the HFE protein modulates uptake of transferrin-bound iron by undifferentiated intestinal crypt cells, thereby programming the absorptive capacity of enterocytes derived from these cells. However, over the years, this ‘crypt model’ as the sole explanation of unneeded iron entering the circulation became controversial. Indeed, recently a normal iron metabolism was described despite the lack of HFE gene expression in the duodenum. In 2003, mice studies by Nicolas et al. suggested that it is mainly the failure of hepcidin induction that contributes to the pathogenesis of HH. Hepcidin has been shown to regulate iron homeostasis by internalisation and subsequent degradation of ferroportin, a major cellular iron exporter protein in the duodenal villi cells and macrophages, thereby suppressing iron uptake and release, respectively. Absent or very low hepcidin concentrations lead to a juvenile onset of the clinical iron overload disease, whereas moderately decreased hepcidin concentrations, in case of mutations in the HFE gene, lead to relatively low and late onset of iron overload disease.

**CLINICAL SIGNS AND SYMPTOMS**

In 2000 an expert group described HFE-related HH as follows: ‘HH is an inherited disorder resulting from an inborn error of iron metabolism which leads to progressive loading of parenchymal cells in the liver, pancreas and heart. In its fully developed stage organ structure and function are impaired’. Early clinical symptoms are described to encompass weakness, joint pain, palpitations and abdominal pain, whereas massive iron overload will ultimately lead to arthritis, severe fatigue, chronic abdominal pain, liver enzyme elevations, liver cirrhosis, primary liver cancer, diabetes mellitus, hypopituitarism, hypogonadism, congestive heart failure, cardiac dysrhythmias, increased skin pigmentation and an increased risk of certain bacterial infections. All symptoms are relatively nonspecific, making it difficult to recognise them as being related to iron overload. In addition the clinical penetrance of the HFE gene mutations is very variable. Until now searches for additional gene mutations that may identify patients at increased risk of developing clinical manifestations of haemochromatosis have not been successful.

**DIAGNOSIS OF IRON OVERLOAD**

Elevated iron parameters in the serum, i.e. serum transferrin saturation (TS) and serum ferritin (SF) are a strong indication for altered iron metabolism (figure 1). In the literature various reference ranges are mentioned, probably due to differences in the populations examined and lack of standardisation of especially serum ferritin analysis. A serum transferrin saturation above 45%, in combination with an elevated SF level, is highly suggestive...
for increased body iron levels. However, abnormal values can be found in the presence of other pathology, including liver diseases and alcohol abuse. Homozygosity for the C282Y mutation or the combined C282Y/H63D genotype in the HFE gene analysis confirms the HH diagnosis.

The traditional gold standard for diagnosing iron overload is a liver biopsy, although it is generally only required for diagnosis in the presence of comorbidities and for prognosis and management when serum ferritin levels exceed 1000 µg/l. Hepatic magnetic resonance imaging (MRI) provides a noninvasive approach to semi-quantify the amount of liver iron. The severity of iron overload can also be calculated from the number of phlebotomies required to deplete iron stores.

TREATMENT OF HEREDITARY HAEMOCROMATOSIS

The treatment of HH consists of venesection, as described by Davis. It is safe, inexpensive, and appears to be effective, although this has never been proved. With the removal of 500 ml of blood, about 200 to 250 mg of iron is withdrawn from the body. Venesection is started when the SF levels are consistently above the upper limit of the reference range, pointing to body iron excess. Meanwhile, other causes of increased SF must be eliminated. Weekly phlebotomies are performed to withdraw excessive amounts of iron, followed by yearly measurement of the serum ferritin and when necessary maintenance phlebotomies to maintain low body iron stores.

Erythrocytapheresis might be an attractive alternative but more studies are awaited to assess its (cost) effectiveness in comparison with venesection. Next to venesection, dietary advice has been described to be beneficial, including moderation of alcohol intake and avoidance of iron, vitamin C supplements and uncooked seafood. Consumption of black tea with meals has been reported to decrease iron absorption by formation of nonabsorbable iron complexes.

FROM EARLY DIAGNOSIS AND TREATMENT TO DEATH PREVENTION

Despite the high frequency of the C282Y mutation and the obvious iron overload in a subset of patients, the clinical diagnosis of HH is easily overlooked and delayed until irreversible organ damage has developed, as early symptoms are relatively nonspecific. Even more advanced complications are not always recognised as symptoms of HH, unless specifically looked for. This is underlined by the recent findings of Powell et al. Through assessment of disease manifestation by clinical examination and liver biopsy in their population of asymptomatic C282Y homozygous subjects, they found that hepatic iron overload was already present in 56% of the males and 35% of the female subjects. Moreover, one or more unrecognised HH-related disease conditions (arthropathy, diabetes mellitus, hepatomegaly, hypogonadism or cardiac arrhythmia) were present in 30% of the males and 12% of the females. This supports the statement that screening is mandatory for early detection of HFE-related iron overload to prevent organ failure and death.

To reappraise in general terms the indication for and attitude to screening Whitby restated the principles of early disease detection set up by Wilson and Jungner (table 1). Many reports have been written on the feasibility of early screening on HH in the general population. Indeed, HFE-related HH meets important criteria as described by Wilson & Jungner, and Whitby: A recognisable latent or early stage, a suitable test for examination, facilities for diagnosis and treatment and an accepted treatment.
One important question that remains unanswered: Is HH indeed an important health problem, for the community, and for the individual? At first it was assumed that all C282Y homozygous individuals would eventually develop iron overload resulting in tissue damage and disease. But selection bias, differences in case definition and population characteristics led to different findings. Some authors found haemochromatosis-related disease in a high percentage of C282Y homozygous individuals, whereas others barely found any penetrance of the HFE gene mutations.

Some large and controlled studies reported that a significant proportion of the C282Y homozygotes had no symptoms of disease at all, questioning the importance of the health problem.

Another principle of screening still not profoundly resolved is statement 8 added by Whitby (table 1): Treatment at the presymptomatic, borderline stage of a disease should favourably influence its course and prognosis. The cost of case finding (which would include the cost of case finding and treatment) needs to be economically balanced in relation to possible expenditure on medical care as a whole.

Case finding should be a continuing process and not a ‘once and for all’ project.

Table 1. Restatement of the Wilson and Jungner principles for mass screening programmes (World Health Organization, 1968)

<table>
<thead>
<tr>
<th>Statement</th>
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<tbody>
<tr>
<td>1. The condition being sought should be an important health problem, for the individual and the community</td>
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<tr>
<td>2. There should be an acceptable form of treatment for patients with recognisable disease</td>
</tr>
<tr>
<td>3. The natural history of the condition, including its development from latent to declared disease, should be adequately understood</td>
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<tr>
<td>4. There should be a recognisable latent or early symptomatic stage</td>
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<tr>
<td>5. There should be a suitable screening test or examination for detecting the disease at the latent or early symptomatic stage, and this test should be acceptable to the population</td>
</tr>
<tr>
<td>6. The facilities required for diagnosis and treatment of patients revealed by the screening programme should be available</td>
</tr>
<tr>
<td>7. There should be an agreed policy on whom to treat as patients</td>
</tr>
<tr>
<td>8. Treatment at the presymptomatic, borderline stage of a disease should favourably influence its course and prognosis</td>
</tr>
<tr>
<td>9. The cost of case finding (which would include the cost of case finding and treatment) needs to be economically balanced in relation to possible expenditure on medical care as a whole</td>
</tr>
<tr>
<td>10. Case finding should be a continuing process and not a ‘once and for all’ project</td>
</tr>
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</table>

One important question that remains unanswered: Is HH indeed an important health problem, for the community, and for the individual? At first it was assumed that all C282Y homozygous individuals would eventually develop iron overload resulting in tissue damage and disease. But selection bias, differences in case definition and population characteristics led to different findings. Some authors found haemochromatosis-related disease in a high percentage of C282Y homozygous individuals, whereas others barely found any penetrance of the HFE gene mutations.

Some large and controlled studies reported that a significant proportion of the C282Y homozygotes had no symptoms of disease at all, questioning the importance of the health problem.

Another principle of screening still not profoundly resolved is statement 8 added by Whitby (table 1): Treatment at the presymptomatic, borderline stage of a disease, early treatment, should favourably influence the course and prognosis of the disease. In other words it should be more effective started early than started later in the disease development and/or clinical phase.

How to decide which population is to be screened? Searching for individuals with an elevated risk of HH can be performed at three population levels: i) clinical examination of individuals with symptoms pointing to HH, i.e. targeted screening or case detection; ii) screening the families of patients in whom the clinical diagnosis of HH has been made; and iii) population screening (figure 1).

Ad i) Case detection
Medical examination of individuals with symptoms pointing to HH is a very direct way of detecting patients with HH. However, despite the high frequency of C282Y homozygosity in Northern European countries, it can be assumed that the clinical disease is under diagnosed, possibly due to the misunderstanding on the part of physicians that the diagnosis should only be considered if skin bronzing / hyperpigmentation, diabetes mellitus and hepatic cirrhosis are present. Furthermore, unfamiliarity with the existence of the disease and scepticism about the prevalence are a serious barrier to accepting an effective screening for HH. Therefore, it is important to make physicians more aware of the nature of HFE-related HH, e.g. the gene mutation frequency, its clinical penetrance and phenotypic expression, and also of the diagnostic pathway and therapeutic options when choosing this type of screening. Implementation of a guideline for physicians on the targeted detection of HH in an early, symptomatic, stage could be beneficial. Jacobs et al. studied the impact of such a guideline. It led to an increased awareness for HH, but at the cost of an increased rate of false-positive newly diagnosed HH patients. Of the patients eligible for HH, 70% were still not tested.

Taken together, this screening strategy of case detection has its shortcomings for early disease detection.

Ad ii) Family screening
In family screening first-degree relatives of C282Y homozygous patients with clinically detected HFE-related HH are screened for HH. After all, these family members are at relatively high risk: there is a 25% risk of siblings being homozygous. They are likely to share genetic and environmental factors with the clinically positive proband, which may engrave phenotypic expression of HH. From a theoretical point of view this screening strategy has a potentially increased detection rate as well as higher effectiveness of early intervention.

Ad iii) Population screening
In comparison with family screening, population screening offers the possibility of an even earlier and larger-scale detection of HFE-related HH. However, health-threatening symptoms have been shown to occur in only a minority of C282Y homozygotes, making population screening not the first option of HH screening given the low penetrance for cirrhosis of the liver of 2% found by Beuter and 5% found by Powell.

FUTURE INTERVENTION

HFE-related HH is a recognised clinical entity, with variable clinical penetrance. Screening and detecting those
individuals at high risk of iron overload, before irreversible damage evolves, is likely to prevent organ detriment and death. From all the mentioned screening options, family screening is likely to be the most appropriate approach. However, before starting screening programmes questions remain to be answered: Do C282Y homozygous individuals have a relevant health problem? Which individuals are at risk to develop HFE-related iron overload and its accompanied disease? Is screening for these individuals cost-effective? To get an answer to these questions the Dutch HEmochromatosis FAmily Study (HEFAS) was initiated. From 224 probands homozygous for the C282Y mutation and presenting with clinically recognised symptoms of HH and 735 of their first-degree family members a large set of data has been collected, with regard to demographics, lifestyle (smoking, use of alcohol, diet), health, disease, and family structure, including familial death rate. Additionally iron parameters and HFE genotype were collected or determined. These data are currently being analysed; preliminary results are reported in an accompanying paper in this issue (80). They can give diagnostic information, and evidence for or against an association of HFE-linked hemochromatosis with other diseases in the family (9). In conclusion, there are changing views concerning the penetrance of HFE mutations. The need for diagnosing HH early is a challenge to develop appropriate screening strategies for prevention of iron overload-related tissue damage in individuals at risk.

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Morbidity and mortality in first-degree relatives of C282Y homozygous probands with clinically detected haemochromatosis compared with the general population: the HEmochromatosis FAmily Study (HEFAS)


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ABSTRACT

Background: Family screening has been suggested as a sophisticated model for the early detection of HFE-related hereditary haemochromatosis (HH). However, until now, controlled studies on the morbidity and mortality in families with HH are lacking.

Methods: Data on iron parameters, morbidity and mortality were collected from 224 Dutch C282Y-homozygous probands with clinically overt HH and 735 of their first-degree family members, all participating in the HEmochromatosis FAmily Study (HEFAS). These data were compared with results obtained from an age- and gender-matched normal population. HEFAS and controls filled in similar questionnaires on demographics, lifestyle factors, health, morbidity and mortality.

Results: A significantly higher proportion of the HEFAS first-degree family members reported to be diagnosed with haemochromatosis-related diseases: 45.7% vs 19.4% of the matched normal population (McNemar p<0.001). Mortality among siblings, children and parents in the HEFAS population was similar to that in the relatives of the matched controls.

Conclusion: In this study we show that morbidity among first-degree family members of C282Y-homozygous probands previously diagnosed with clinically proven HH is higher than that in an age- and gender-matched normal population. Further studies are needed to definitely connect these increased morbidity figures to increased prevalence of the C282Y mutated HFE-gene and elevated serum iron indices.

KEYWORDS

Family, hereditary haemochromatosis, HFE, morbidity, mortality

INTRODUCTION

HFE-related hereditary iron overload is characterised by iron deposition in parenchymal organs.1-4 Early detection and phlebotomy prevent tissue damage and result in long-term survival similar to that in the general population.4-6 Of Northern European patients diagnosed with hereditary haemochromatosis (HH), 80% appear to be homozygous for the C282Y mutation in the HFE gene. The carrier frequency of this C282Y mutation in the general Caucasian population is estimated to be as high as one in every ten
persons. Altogether, this would favour population screening to prevent disease-related morbidity. Recently, however, it was shown that not all C282Y-homozygous individuals develop symptoms of iron overload disease, debating the penetrance of the HFE-gene mutations. Therefore, family screening has been suggested, since this has proven efficacy in the detection of latent homozygotes for frequent recessive mutations. Nevertheless, until now, one important item in the World Health Organisation (WHO) guidelines for screening for disease, published in 1968, has remained unanswered for HH-related family screening: Is HH in these families an important health problem? However, to date, to our knowledge there is no such a study that has extensively compared the morbidity and mortality rate for the two populations.

Data for the HH families were obtained from the HEmochromatosis FAmily Study (HEFAS), which was designed to collect clinical, biochemical, genetic and mortality data from Dutch C282Y-homozygous probands as well as from their first-degree relatives. All probands in the HEFAS had been previously diagnosed with symptomatic HFE-related HH. The controls were recruited from the Nijmegen Biomedical Study (NBS), a population-based survey conducted among 22,400 inhabitants of the Dutch city of Nijmegen in 2002-2003.

**STUDY POPULATION AND METHODS**

**HEFAS population**

For this study, 280 probands diagnosed with symptomatic HFE-related HH from five different medical centres in the Netherlands were actively approached (figure 1). The local medical ethics committees of each of these centres approved the study protocol before the start of the study. A total of 224 probands participated. They provided the HEFAS with names and addresses of 972 first-degree family members (defined in this study as biological parents, full siblings, and biological children), 18 years of age and older, of whom 735 met the inclusion criteria. Participants were included from May 2003 until August 2005.

**Inclusion**

Only subjects who gave written informed consent were included in the study. Probands had to be at least 18 years old and to have been clinically diagnosed with

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**Figure 1. Flowchart of the invited and participating probands, the accompanying family members and their available data**

Probands were classified as ‘defined proband’ when symptoms consistent with hereditary haemochromatosis, C282Y homozygosity and iron overload were present, confirmed by either the plasma iron parameters, iron levels in a liver biopsy or the number of phlebotomies. Laboratory data: iron parameters (transferrin saturation, serum ferritin) and HFE genotyping. Participating hospitals: Atrium Medical Centre, Heerlen/Brunssum; Radboud University Nijmegen Medical Centre; Rijnstate Hospital Arnhem; University Medical Centre Groningen and University Medical Centre Utrecht.
C282Y-homozygous HH. The iron overload had to be confirmed by initial serum ferritin (SF) and transferrin saturation (TS) values exceeding the thresholds of SF ≥280 µg/l for men, SF >80 µg/l for women under the age of 50, SF ≥180 µg/l for women ≥50 years and TS >50% for both men and women. When either one or both pretreatment plasma iron parameters were unavailable, the presence of iron overload was alternatively confirmed by previously performed liver biopsy (grade 3 iron deposition according to Sindram) or by the number of phlebotomies required to normalise SF (males ≥22 phlebotomies = 5 g chelatable iron; females ≥13 phlebotomies = 3 g chelatable iron).15

Questionnaires
All participants were asked to fill in a questionnaire containing a large number of questions on demographics, lifestyle (smoking, use of alcohol, diet), health status, general medical history, morbidity, medical history for HH, implementation of family screening, legal, psychological and societal implications, and family structure including familial mortality.

Laboratory data
Data on the included probands and family members were extracted from the medical records of the participating hospitals. Information on iron parameters (TS and SF) and liver biopsy of the participants was obtained only at the time of diagnosis of HH or the time of screening for HH, whereas data on the number of phlebotomies were also collected at points in time after the initial investigations. When incomplete, the physician involved in the diagnosis and treatment of the participants was asked to provide the HEFAS team with these data. Finally, when the data remained deficient or the subjects declared that they had never been tested for HH, participants were offered counselling and blood testing by their general practitioner (GP).

Iron parameters for HEFAS were collected by several clinical laboratories. The TS and SF were quantified using validated, standardised, routine laboratory methods. The amount of iron in the liver biopsies was assessed semi-quantitatively.15

The Nijmegen Biomedical Study (NBS)
The Nijmegen Biomedical Study (NBS) is a population-based survey conducted among inhabitants of the city of Nijmegen in 2002-2003.14 Nijmegen is a town in the eastern part of the Netherlands with 156,000 inhabitants, approximately 87% of Caucasian descent. The aim was to obtain a representative sample of the normal population in the Netherlands that could be used as a universal control population for a wide range of medical studies. Randomly selected, age- and gender-stratified inhabitants of Nijmegen (n=22,452) were taken from the population registry and received an invitation to fill in a postal questionnaire on lifestyle and medical and family history that was comparable with the HEFAS questionnaire. Approval to conduct the NBS was obtained from the Institutional Review Board of the Radboud University Nijmegen Medical Centre (RUNMC). The response to the questionnaire was 41.7% (n=9371). In addition, 69.1% of these responders donated 30 ml of blood each for DNA isolation, serum and plasma (n=6473). Analysis of the plasma iron parameters was performed in the Departments of Clinical Chemistry and Chemical Endocrinology of the RUNMC.

Statistical methods
In order to compare the data from HEFAS with those of the general population, a one-to-one age- and gender-matched sample was randomly drawn from the 9371 participants in the NBS. The cut off values at 65% of the scales of general mental health, physical functioning, vitality16 and fatigue17 were used for further evaluation.

Haemochromatosis-related medication use was calculated by counting the use of (i) analgesics, (2) antihypertensive drugs and (3) cardiovascular medication (i.e. use of at least one of the following: antihypertensive drugs, cardiovascular drugs and diuretics), for each person resulting in a score that ranged from 0-3. Similarly, the number of haemochromatosis-related diseases was calculated by counting the presence of (i) diabetes mellitus, (2) liver disease, (3) rheumatism, (4) fatigue (score ≥8) and (5) cardiovascular disease, for each person resulting in a score that ranged from 0-5. Haemochromatosis-related medication use (yes, no) and haemochromatosis-related morbidity (yes, no), were used for further evaluation.

We compared HEFAS and NBS with regard to i) the percentage of elevated iron parameters using local reference values for each of the participating laboratories, and ii) the absolute values of iron parameters using data obtained in only one single laboratory, that of the RUNMC (ca. 25%). The rationale for choosing this laboratory is that the sera of all participants in the NBS were analysed at this location. Prior to the analysis, both the actual iron parameters and the body mass index (BMI) were transformed logarithmically to improve skewness. Differences in the means of the logarithmically transformed data between the HEFAS and the age- and gender-matched sample from the NBS were tested for statistical significance using the t-test for paired data. The back-transformed mean differences with the 95% confidence intervals (95% CI) are presented. Differences in single proportions between the HEFAS probands and the age- and gender-matched sample from the NBS were tested for statistical significance using McNemar’s test. The percentage differences between the HEFAS and the NBS samples were calculated together with the 95% CI that takes into account the matched pair design. Because p values and the corresponding confidence intervals are then univocally

related, it is not necessary to present both; therefore, only the differences with the corresponding confidence intervals are presented here. As this is a descriptive study, no corrections for multiple comparisons were performed.

The mortality within HEFAS families, as reported by the probands, was compared with the mortality in the families of the matched NBS participants. Differences in mortality between the HEFAS and the matched NBS sample were tested for statistical significance using Fisher’s exact test, separately among parents, siblings and children.

A two-tailed p value <0.05 was considered statistically significant. Analyses were performed using SAS version 8.2.

RESULTS

Study population

Of the 280 probands, 224 (80.0%) filled in the questionnaires and the informed consent forms (figure 1). These 224 probands provided names and addresses of 972 FDFM, ≥18 years of age, of whom 735 (75.6%) were included. Of these 735 relatives, 155 reported to have been diagnosed with HH in the past. Figure 1 shows that 100% of the included probands gave permission for analysis of their laboratory results, whereas 17 (2%) family members did not approve retrieval of laboratory data from their records or agree to additional withdrawal of blood for laboratory tests if data were missing.

Table 1 shows the size and structure of the families of the included HEFAS probands. Twenty-four (10.7%) of the 224 probands who entered the study had more than five participating siblings, whereas 78 (34.8%) had no participating siblings. Four probands had more than five children included in the study, whereas 105 probands had no participating children. In total, this study involved 224 probands, 428 siblings, 241 children and 66 parents.

Demographics and lifestyle

Table 2 shows the results of the self-reported demographics and lifestyle characteristics of the FDFM and the matched NBS participants. The median age at participation was 48 years (range: 18-97 years), and 56.7% of the participants were women. Because of the matched design these values are identical in both studies.

<table>
<thead>
<tr>
<th>Table 1. Size and structure of the families of the HEmochromatosis FAmily Study (HEFAS) probands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siblings</td>
</tr>
<tr>
<td>Children:</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>44</td>
</tr>
<tr>
<td>105</td>
</tr>
<tr>
<td>Both parents</td>
</tr>
<tr>
<td>18</td>
</tr>
<tr>
<td>Father or mother</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>No parents</td>
</tr>
<tr>
<td>70</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Characteristics of the first-degree family members of the HEFAS probands and of the age- and gender-matched NBS participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEFAS</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Demographics:</td>
</tr>
<tr>
<td>• Age at participation (years)</td>
</tr>
<tr>
<td>• Men</td>
</tr>
<tr>
<td>• Education (≥secondary)</td>
</tr>
<tr>
<td>• Household (single with or without children)</td>
</tr>
<tr>
<td>• Paid job (≥32 hrs/week)</td>
</tr>
<tr>
<td>Lifestyle:</td>
</tr>
<tr>
<td>• Alcohol (&gt;2 units/day)</td>
</tr>
<tr>
<td>• Smoking (ever)</td>
</tr>
<tr>
<td>Blood loss:</td>
</tr>
<tr>
<td>• Blood donation (never)</td>
</tr>
<tr>
<td>• ∘Menarche (≤12 years)</td>
</tr>
<tr>
<td>• ∘Pregnancies (&gt;3)</td>
</tr>
</tbody>
</table>

HEFAS = HEmochromatosis FAmily Study, encompassing probands with clinically overt HFE-related haemochromatosis and their first-degree family members; NBS = Nijmegen Biomedical Study, consisting of a representative sample of the Dutch population; CI = confidence interval, using the matched pair design; n.a. = not applicable, because the first-degree family members of the HEFAS and the NBS participants are matched one-to-one by age and gender. "Number of matched pairs with valid data; "the increase from HEFAS to NBS, using the matched pair design. JACOBs, et al. Morbidity in families with haemochromatosis.
Table 3. General health, medication, morbidity and iron parameters in the first-degree family members of the HEFAS probands and of the age- and gender-matched NBS participants

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HEFAS</th>
<th>NBS</th>
<th>HEFAS - NBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index (kg/m²)</td>
<td>Total Median (range)/n (%)</td>
<td>Total Median (range)/n (%)</td>
<td>Total Difference (95% CI)</td>
</tr>
<tr>
<td>General health:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Exercise (≤1 hr/week)</td>
<td>415 109 (26.3)</td>
<td>412 151 (36.7)</td>
<td>250 -4.4 (-12.0; -3.2)</td>
</tr>
<tr>
<td>• Health (&gt;2)‡</td>
<td>722 70 (11.4)</td>
<td>733 60 (9.1)</td>
<td>720 6.5 (2.2; 10.9)</td>
</tr>
<tr>
<td>• General mental health last 4 weeks (≤23)§</td>
<td>684 339 (49.6)</td>
<td>697 461 (51.8)</td>
<td>650 -1.7 (7.1; 1.7)</td>
</tr>
<tr>
<td>• Physical functioning at this moment(23)##</td>
<td>656 108 (16.5)</td>
<td>686 72 (11.5)</td>
<td>617 6.0 (2.5; 9.5)</td>
</tr>
<tr>
<td>• Vitality last 4 weeks (≤17) ¥¥</td>
<td>680 376 (55.3)</td>
<td>701 325 (46.4)</td>
<td>649 9.1 (3.7; 14.4)</td>
</tr>
<tr>
<td>• Analgesics</td>
<td>627 321 (51.2)</td>
<td>691 285 (41.2)</td>
<td>593 9.8 (4.1; 15.4)</td>
</tr>
<tr>
<td>• Antihypertensive drugs</td>
<td>654 146 (22.3)</td>
<td>690 109 (16.5)</td>
<td>574 2.8 (1.0; 4.6)</td>
</tr>
<tr>
<td>• Antirheumatic drugs</td>
<td>601 63 (10.5)</td>
<td>673 35 (5.2)</td>
<td>576 5.9 (2.9; 9.0)</td>
</tr>
<tr>
<td>• Cardiovascular drugs</td>
<td>614 70 (11.4)</td>
<td>681 50 (7.3)</td>
<td>574 4.4 (1.2; 7.5)</td>
</tr>
<tr>
<td>• Diuretics</td>
<td>606 73 (12.0)</td>
<td>683 61 (9.3)</td>
<td>572 3.2 (0.1; 6.2)</td>
</tr>
<tr>
<td>• Folic acid</td>
<td>583 67 (11.5)</td>
<td>655 61 (9.3)</td>
<td>531 2.4 (1.0; 5.8)</td>
</tr>
<tr>
<td>• Lipid-lowering drugs</td>
<td>614 57 (9.3)</td>
<td>682 48 (7.0)</td>
<td>569 9.0 (2.7; 5.2)</td>
</tr>
<tr>
<td>• Iron supplements</td>
<td>718 87 (12.1)</td>
<td>674 141 (20.9)</td>
<td>659 -2.6 (-6.8; 1.6)</td>
</tr>
<tr>
<td>• Tranquillizers</td>
<td>618 148 (24.0)</td>
<td>696 150 (21.6)</td>
<td>590 3.0 (0.1; 5.8)</td>
</tr>
<tr>
<td>• (Multi)vitamin preparations</td>
<td>613 221 (36.0)</td>
<td>675 199 (29.5)</td>
<td>570 6.0 (0.5; 11.5)</td>
</tr>
<tr>
<td>• Vitamin B complex</td>
<td>593 139 (23.4)</td>
<td>668 124 (18.6)</td>
<td>542 5.5 (1.0; 10.2)</td>
</tr>
<tr>
<td>• Vitamin C complex</td>
<td>601 197 (32.8)</td>
<td>670 174 (26.0)</td>
<td>556 7.9 (2.7; 13.1)</td>
</tr>
<tr>
<td>• Haemochromatosis-related medication</td>
<td>677 421 (62.2)</td>
<td>708 345 (49.2)</td>
<td>652 13.3 (8.2; 18.4)</td>
</tr>
<tr>
<td>Morbidity¥:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Anaemia</td>
<td>620 99 (16.0)</td>
<td>674 90 (11.4)</td>
<td>575 3.0 (-1.1; 7.0)</td>
</tr>
<tr>
<td>• Cancer</td>
<td>621 35 (5.6)</td>
<td>683 48 (7.0)</td>
<td>584 -1.4 (-4.1; 1.4)</td>
</tr>
<tr>
<td>• Cardiovascular disease</td>
<td>654 65 (10.5)</td>
<td>685 28 (4.1)</td>
<td>582 5.3 (2.7; 8.3)</td>
</tr>
<tr>
<td>• Cerebrovascular accident</td>
<td>604 23 (3.8)</td>
<td>657 9 (1.3)</td>
<td>551 1.4 (-0.1; 3.0)</td>
</tr>
<tr>
<td>• Diabetes mellitus</td>
<td>620 25 (4.0)</td>
<td>685 31 (4.6)</td>
<td>574 0.4 (-1.8; 2.5)</td>
</tr>
<tr>
<td>• Fatigue (≥18)**</td>
<td>688 90 (13.1)</td>
<td>674 87 (12.1)</td>
<td>659 2.6 (-2.2; 7.2)</td>
</tr>
<tr>
<td>• Hypercholesterolaemia</td>
<td>614 47 (7.7)</td>
<td>681 48 (7.2)</td>
<td>674 1.5 (-1.0; 3.1)</td>
</tr>
<tr>
<td>• Hypertension</td>
<td>604 22 (3.6)</td>
<td>669 28 (4.2)</td>
<td>557 0.5 (-3.0; 4.1)</td>
</tr>
<tr>
<td>• Osteoporosis</td>
<td>638 31 (5.1)</td>
<td>669 17 (2.5)</td>
<td>563 3.2 (1.0; 5.4)</td>
</tr>
<tr>
<td>• Rheumatism</td>
<td>620 199 (32.4)</td>
<td>668 28 (4.1)</td>
<td>596 1.4 (-0.1; 3.0)</td>
</tr>
<tr>
<td>• Surgery</td>
<td>612 47 (7.7)</td>
<td>677 25 (3.7)</td>
<td>651 1.4 (-0.1; 3.0)</td>
</tr>
<tr>
<td>• Thyroid disease</td>
<td>610 23 (3.6)</td>
<td>671 9 (1.3)</td>
<td>563 3.2 (1.0; 5.4)</td>
</tr>
<tr>
<td>• Haemochromatosis-related diseases (diabetes mellitus, liver disease, rheumatism, fatigue and cardiovascular disease)</td>
<td>652 238 (45.7)</td>
<td>673 131 (21.9)</td>
<td>599 25.7 (20.9; 30.5)</td>
</tr>
<tr>
<td>Iron parameters*:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Serum transferrin saturation &gt;50%</td>
<td>599 176 (29.4)</td>
<td>494 21 (4.2)</td>
<td>405 25.3 (20.5; 30.1)</td>
</tr>
<tr>
<td>• Serum ferritin above normal (µmol/l)‡‡</td>
<td>487 198 (40.7)</td>
<td>409 106 (21.2)</td>
<td>353 16.3 (9.7; 23.3)</td>
</tr>
<tr>
<td>• Serum transferrin saturation (%)§§</td>
<td>207 18.4 (3.2-107.3)</td>
<td>135 29.5 (4.8-97.7)</td>
<td>135 33.2 (21.4; 53.2)</td>
</tr>
<tr>
<td>• Serum ferritin (µmol/l)‖</td>
<td>207 119.0 (4.0-2308)</td>
<td>137 131.9 (6.6-4737)</td>
<td>137 32.4 (7.4; 63.1)</td>
</tr>
</tbody>
</table>

HEFAS = HEmochromatosis FAmily Study, encompassing probands with clinically overt HFE-related haemochromatosis and their first-degree family members; NBS = Nijmegen Biomedical Study, consisting of a representative sample of the Dutch population; CI = confidence interval, using the matched pair design. Number of matched pairs with valid data. Increase from HEFAS to NBS, using the matched pair design. 1: feeling good to 5: feeling bad; 5: bad mental health to 30: good mental health, using the SF-36 health survey score. 16 16: low vitality to 24: high vitality, using the SF-36 health survey score. 16 Self-reported diagnosis of morbidity made by a physician. 14: fatigue absent to 24: fatigue present, using the shortened fatigue questionnaire score. 17 At time of being tested for hereditary haemochromatosis. 18 Serum ferritin above the local upper reference value; only participants tested in the Radboud University Nijmegen Medical Centre.
The number of participants with at least secondary education was significantly lower in the FDFM of the HEFAS population compared with the matched NBS participants (HEFAS% minus NBS%: -9.9%) while the percentage of participants with paid jobs was similar for both populations. The HEFAS FDFM reported a significantly lower alcohol intake compared with the NBS controls (>2 units alcohol/day, HEFAS%-NBS%: -8.3%). Yet, the smoking behaviour of both groups was similar.

**General health, medication, morbidity and iron parameters**

Table 3 summarises the general health, medication, morbidity and iron parameters of the FDFM in the HEFAS population and the age- and gender-matched NBS participants. The median BMI of the HEFAS FDFM was slightly but significantly higher than that of the population-based controls of the NBS (HEFAS%-NBS%: 17%, 95% CI 0.1-2.4%). The HEFAS FDFM reported significantly more hours of exercise during the week, they also felt better (health) but had a lower level of physical functioning and vitality. Significantly more FDFM of the HEFAS population were on antihypertensive drugs (HEFAS%-NBS%: 8.8%) analgesics (HEFAS%-NBS%: 9.8%), antirheumatic drugs (HEFAS%-NBS%: 5.9%) and cardiovascular drugs (HEFAS%-NBS%: 4.4%). Iron supplements were less frequently taken by the HEFAS FDFM, than by the matched NBS participants (HEFAS%-NBS%: -9.0%). Cardiovascular disease, hypercholesterolaemia and hypertension were reported significantly more frequently by the FDFM of the HEFAS population than by the participants in the control population (table 3). Fatigue (HEFAS%-NBS%: 5.9%), liver disease (HEFAS%-NBS%: 3.2%), osteoporosis (HEFAS%-NBS%: 4.2%) and especially rheumatism (HEFAS%-NBS%: 24.6%) were also diagnosed significantly more frequently among the FDFM of the HEFAS population. In contrast, diabetes mellitus and infertility were diagnosed with similar frequencies in both populations (table 3). The iron parameters TS and SF were both significantly more often elevated in the FDFM of the HEFAS probands compared with the matched NBS participants, with a difference between HEFAS and NBS for TS of 25.3% and for SF of 16.5% (table 3). Similarly, the relative differences in the absolute values of TS and SF between the FDFM of the HEFAS and the matched NBS participants were 37.1 and 32.4%, respectively, using only the samples measured in the RUNMC.

**Figure 2.** The amount of haemochromatosis-related medication use and the number of haemochromatosis-related diseases in the first-degree family members of the HEFAS probands (black) and of the age- and gender-matched NBS participants (grey).

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**Table 4. Mortality among first-degree family members of both HEFAS probands and age- and gender-matched NBS participants**

<table>
<thead>
<tr>
<th></th>
<th>HEFAS</th>
<th></th>
<th>NBS</th>
<th></th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Families</td>
<td>n</td>
<td>Deceased</td>
<td>n (%)</td>
<td>Families</td>
</tr>
<tr>
<td>Parents</td>
<td>224</td>
<td>427</td>
<td>299 (70.0)</td>
<td>224</td>
<td>421</td>
</tr>
<tr>
<td>Siblings</td>
<td>224</td>
<td>709</td>
<td>93 (13.1)</td>
<td>224</td>
<td>752</td>
</tr>
<tr>
<td>Children</td>
<td>224</td>
<td>414</td>
<td>8 (1.9)</td>
<td>224</td>
<td>372</td>
</tr>
</tbody>
</table>

Families = number of families reported by the HEFAS probands or the age- and gender-matched NBS participants; n = number of family members reported by the proband or the age- and gender-matched NBS participant, respectively. P value for difference in proportion between the HEFAS and the NBS group, using Fisher's exact test.
medication, compared with the NBS participants, i.e. a difference between HEFAS and NBS of 13.3%. Similarly, a significantly higher percentage of FDFM reported to be diagnosed with one or more disease, i.e. a difference between HEFAS and NBS of 25.7%.

**Mortality**

All 244 HEFAS probands provided data on the mortality of their first-degree relatives. The probands provided information on 427 parents, of whom 70.0% (n=299) had died by the end of our study (table 4). These mortality figures did not differ significantly from the reported 73.6% (n=310) deceased parents of the 224 age- and gender-matched NBS participants (p=.025). Similarly, the mortality among the siblings and children of the HEFAS families did not differ significantly from that of the NBS families.

**DISCUSSION**

Family screening can be a sophisticated model for screening of HH. However, to date, to the best of our knowledge controlled studies on morbidity and mortality in families with HH are lacking. Indeed, the present study reveals more haemochromatosis-related diseases in the HEFAS population compared with the general population. In contrast, the mortality in the HEFAS population was not significantly higher than in the normal population. Earlier studies have already described fatigue, weakness and arthropy as being related to HFE gene mutations, whereas diabetes mellitus, abnormal liver function tests, impotence, hypothyroidism, cardiomyopathy and hepatocellular carcinoma were mentioned as some of the more specific, organ-related problems leading to increased morbidity and mortality.1-3 If HH were diagnosed and treated in time, tissue damage could be prevented and a long-term survival similar to that in the general population could be achieved.2-6 Nevertheless, recent studies claim that although some iron-overloaded patients with homozygosity for the C282Y mutation in the HFE gene have a high and probably preventable morbidity, even more subjects with this genotype had no symptoms at all.8-11 Moreover, studies performed in several European countries could not detect significant differences in the prevalence of untreated homozygotes among elderly populations compared with younger groups.8-11 This cast doubt on the adequacy of presymptomatic population screening. Thus, family screening was suggested as it was thought to increase the chances to find both C282Y homozygosity (theoretically present in 25% of the siblings) and an elevated penetrance of iron overload due to the sharing of iron metabolism modifying genes or environmental factors with the clinically expressing proband. Indeed, focusing on FDFM of C282Y-homozygous patients with clinically overt HH has been shown to produce a significant yield of C282Y-homozygous individuals with high penetrance of iron accumulation, but with an unknown increase of morbidity compared with the normal population.24-24 McCune et al. recently reported that despite the presence of elevated iron parameters, the morbidity among C282Y-homozygous relatives of probands identified by screening a group of blood donors was similar to that of C282Y-homozygous relatives of probands presenting as patients.25 Assuming that the C282Y homozygous blood donors had less morbidity than the probands of identical genotype presenting clinically, this cast doubt on the contribution of the higher penetrance of iron overload within HFE-mutated families and therefore the significance of family screening. In the present study, however, we demonstrated that first-degree relatives of patients with clinically overt HFE-related HH do have a higher morbidity in comparison with the general population. Admittedly, this study was not designed to clarify the factor that is responsible for the observed morbidity differences. It is evident, however, that HEFAS relatives have a higher possibility of being homozygous and heterozygous for the C282Y mutation compared with the normal population. These differences in genetic predisposition are likely to be the cause of the elevated serum iron indices of the HEFAS relatives and the higher incidence of HH-related symptoms. To analyse this further we evaluated the relation between HH-related symptoms and TS, and observed a significant relation between rheumatism and TS%, and a nonsignificant correlation between ‘cardiovascular disease’ and TS%. Thus, additional studies are warranted to definitely attribute the morbidity differences to HFE genotype and iron parameters.

A remarkable finding in this study is the discrepancy between the higher morbidity and similar mortality among the FDFM of the HEFAS probands compared with the matched NBS population. Several explanations can be given. First of all, HEFAS family members as well as their general practitioners may be more aware of the symptoms typical for HH, leading to an advantage in diagnosis and treatment.9 Secondly, the age of the C282Y homozygous siblings (mean 54 years, interquartile range Q1-Q3 47-62 years) might be too low for HFE-related mortality and the study might also comprise too few C282Y homozygous parents to influence the mortality differences between both parental populations. Next to this, other confounding factors that were not measured may have influenced the comparative mortality. It has, for instance, been suggested that C282Y polymorphism may protect against several infectious agents, either by the synthesis of a dysfunctional HFE protein as target receptor for infectious agents, by lowering the iron levels inside macrophages and so inducing resistance to ferrophilic micro-organisms, or
by altering immunological processes, all leading to an advantage in survival.14-20 More recent investigations have demonstrated that non-transferrin-bound iron in the sera of homozygotes and even heterozygotes for the C282Y mutation promoted the adhesion of monocytes to endothelial cells, which may be another advantage of immune defence.19 Furthermore, the HFE gene mutations may provide a survival advantage by ameliorating the iron deficiency seen in another common HLA-defined condition, such as coeliac disease.11 Meanwhile, however, questions on the survival advantage of HFE polymorphism remain.

It should be noted that our study includes a self-reporting questionnaire. Therefore, to diminish a potential registration bias, the questionnaires for both HFAS and NBS participants were identical on the questions evaluated in the present study in that participants were asked to report diseases as diagnosed by their physicians and the fatigue and general health questions were scored by validated questionnaires.

Taken together, this study demonstrates that the morbidity among first-degree relatives of probands with clinically overt HFE-related HH is higher than in the normal population. These findings challenge us to definitely link these morbidity figures to haemochromatosis in future studies.

ACKNOWLEDGEMENTS

We would like to thank the Radboud University Nijmegen (Medical Centre) co-workers Sonja van Oosterhout-van Slageren, data manager, Clinical Chemistry, and Lammy Elving, Internal Medicine, who were of great help in the initial phase of the study, Erny Meij-van Kesteren, Clinical Chemistry, for her work as data manager, Siem Klaver, technician, Clinical Chemistry, for managing the prospective blood sample determinations, Angela van Remortele, genetic counsellor, Anthropogenetics, for counselling the HFAS families and Wim Lemmens, Epidemiology and Biostatistics, for statistical programming. Furthermore, we would like to thank all the enthusiastic Radboud University Nijmegen students and co-workers for retrieving missing data and copying all the available data into the HFAS database: Anke Borgers, Mirrin Dorresteijn, Marja Geurts, Rein Houben, Roel Lucassen, Moniek van de Luitgaarden, Karlijn van Rooijen and Joris Theunissen. We are also grateful to the NBS team of the Radboud University Nijmegen (Medical Centre), specifically Barbara Franke, Anthropogenetics, Lambertus Kiemeney, Epidemiology and Biostatistics, Femmie de Vegt, Epidemiology and Biostatistics, and Martin den Heijer, Endocrinology for sharing information on the NBS database for the present study.

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Incidence of first acute myocardial infarction in the Netherlands

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ABSTRACT

Objectives: To study the incidence of first acute myocardial infarction (AMI) in the Netherlands.

Background: We recently showed that AMI patients can be followed longitudinally within Dutch national medical registrations in a valid way. This makes it possible to provide nationwide incidence estimates of first AMI in the Netherlands.

Methods: New cases of first AMI in the Dutch population in 2000 were identified through linkage of the national hospital discharge register, the population register and the cause of death statistics and included hospitalised first AMI patients and out-of-hospital deaths from first AMI.

Results: 31,777 patients with a first AMI were identified. The incidence (per 100,000 persons per year) increased from 2 in men aged <30 years to 2996 in men aged >90 years. Corresponding figures for women ranged from 1 to 2226. The incidence was higher in men than in women in all age groups, but the male-to-female ratio decreased after the age of 50-59 years. Of all first AMI patients, 40% died before being admitted to a hospital. The proportion of non-hospitalised AMI patients increased with age in men after the age of 40-49 years and in women after the age of 50-59 years. Within the age groups, the proportion of out-of-hospital deaths was similar for men and women.

Conclusion: Our study provides the first nationwide incidence estimates of first AMI in the Netherlands. Expected differences in incidence with regard to age and gender were shown. The large proportion of out-of-hospital deaths reinforces the importance of primary prevention of AMI.

KEYWORDS

Acute myocardial infarction, coronary heart disease, epidemiology, hospital admissions, incidence, medical record linkage, registries

INTRODUCTION

Cardiovascular disease and particularly acute myocardial infarction (AMI) represent a great burden of morbidity and mortality in the Netherlands, as well as in many other Western countries. Information on incidence and mortality of AMI is important for developing and maintaining public health strategies in primary and secondary prevention as well as for monitoring the effects of primary and secondary prevention on incidence and mortality. Information on incidence of acute myocardial infarction tends to come from specifically developed registries, such as the MONICA registries, and from linkage of regional registries. Only a few countries provide nationwide data on the incidence of AMI. In the Netherlands the incidence of AMI is derived from local primary care registries and mortality and hospital discharge rates for AMI were traditionally frequently examined for the Netherlands using national registries. Yet, since it was not possible to track subjects between and within these national registries, the information was of limited value. After we recently showed that hospitalised patients in the Netherlands could be followed longitudinally within Dutch national medical registrations in a valid way, we set out to study the incidence of first AMI encompassing the entire country, with particular emphasis on the proportion not hospitalised.
**METHODS**

**Sources of data**

Data on hospital admissions were derived from the Dutch National Hospital Discharge Register (HDR). Since 1986, all general and university hospitals and most single speciality hospitals are participating in the HDR. There are no private hospitals in the Netherlands that treat patients with AMI. For each hospital admission a new record is created in the HDR, including the following information: date of birth, gender, the numeric part of the postal code (since 1991), hospital-specific patient identification code, type of hospital, admission date and principal diagnosis of the admission. The principal diagnosis is determined at discharge and is in retrospect the main reason for admission. The principal diagnosis is coded using the ninth revision of the International Classification of Diseases (ICD-9-CM). Following individuals over time based on HDR information only is difficult, as different admissions from the same person cannot be recognised adequately. The hospital-specific patient identification code can only be used if patients return to the same hospital, provided that this code is correctly applied. A combination of partial identifying variables (i.e. date of birth, gender and numeric part of postal code) can be used to identify different admissions from the same person provided this combination is unique in the population (it has been shown that 86% of the Dutch population had a unique combination of date of birth, gender and numeric part of postal code on 1 January 1996) and constant over time. The numeric part of postal code, however, can change when patients move (estimated rate of 6% per year). When these patients are subsequently admitted to a hospital that does not register a (usable) hospital-specific patient identification code (19% of the hospitals in 1996) or to another hospital, recognition of these admissions is impossible. Therefore to solve this issue in tracking patients we additionally used information from the Dutch Population Register (PR). This database contains information on all registered persons living in the Netherlands, including date of birth, gender, current address, postal code, nationality and native country (both of registered person and his/her parents). Patients registered in the HDR were identified in the PR using linkage variables ‘date of birth’, ‘gender’ and ‘numeric part of postal code’. When patients moved, their hospital admissions were recognised by using the new postal code registered in the PR. Information on native country in the PR was used to allocate patients in origin categories. Patients whose parents were both born in the Netherlands were classified as native Dutch.

Data on numbers of deaths from AMI in the Netherlands were derived from the national cause of death statistics. These mortality data are virtually complete and comprise both primary and secondary causes of death. Death has been coded using the tenth revision of the International Classification of Diseases (ICD-10).

**Privacy issues**

Linkage of data from the different registers was performed in agreement with the privacy legislation in the Netherlands. Anonymous follow-up was achieved by linking on the variables date of birth, gender and numeric part of postal code. After the linkages, this information was replaced in the database by less specific variables (i.e. age in years and municipal code) to further prevent identification of an individual. All linkages and analyses were performed at Statistics Netherlands in a secure environment ensuring that results meant for publication do not reveal information on individual patients, health care workers or institutions.

**Cohort enrolment**

New cases of first AMI in the Dutch population in 2000 were identified through combining information of the HDR, PR and cause of death statistics and included hospitalised first AMI patients and out-of-hospital deaths from first AMI, as described in detail below.

Between 1 January and 31 December 2000, a total of 24,954 hospital admissions with principal diagnosis AMI (ICD-9-CM code 410 and subcategories) were registered in the HDR (figure 1). This included both patients hospitalised for a first AMI and patients hospitalised for a reinfarction, and both patients discharged alive and patients who died during their hospitalisation. After linkage with the PR, 22,470 admissions from patients with a unique combination of linkage variables in the PR remained in the study population (90%). Thus, each remaining admission linked to only one unique person in the PR (one unique individual in the Netherlands). Admissions linking with more than one person (e.g. administrative twins; two persons with the same date of birth, gender and numeric part of postal code registered in the PR) (7%) or with no person at all (e.g. illegal immigrants or administrative errors) (3%) in the PR were excluded. Selection of the first admission per person of all subsequent admissions of a person occurring during the year 2000 yielded a total of 20,414 patients. Accordingly, 2056 readmissions for an AMI had occurred during the year 2000 (9%). Information on hospital admissions in previous years was obtained by linking of the HDR during the period 1 January 1995 until the (first) admission for an AMI in 2000 to the PR. All uniquely linked hospital admissions with a principal diagnosis of AMI were selected and linked to the above-mentioned cohort of 20,414 patients. Patients with previous admissions for AMI were excluded (1356 patients (7%)). This resulted in the final cohort consisting of 19,058 patients with a first hospitalised AMI in the Netherlands in 2000 (figure 1).
Between 1 January and 31 December 2000, a total of 16,941 deaths with as primary cause of death AMI (ICD-10 code I21) or other ICD-10 codes presumably indicating acute cardiac mortality (I22: subsequent myocardial infarction, I23: current complications following AMI, I24.8: other forms of acute ischaemic heart disease, I24.9: unspecified acute ischaemic heart disease, I46: cardiac arrest, R96: sudden death with unknown cause) were registered in the cause of death statistics (figure 2). This included both patients who died in hospital and those who died out of hospital. Selection of patients who were not already included in the cohort of patients with a first hospitalised AMI in 2000 (as described earlier) yielded a total of 14,578 out-of-hospital deaths. Subsequent selection of the out-of-hospital deaths with a unique combination of linkage variables ‘date of birth’, ‘gender’ and ‘numeric part of postal code’ in the PR, 13,368 out-of-hospital deaths remained in the study population (92%). Information on previous hospital admissions of the out-of-hospital deaths was collected analogously to the collection of information on previous hospital admissions of the patients with a first hospitalised AMI in 2000. Patients with previous admissions with a principal diagnosis of AMI during the period 1 January 1995 until the date of death from an AMI in 2000 were excluded (649 patients (5%)). This resulted in the final cohort consisting of 12,719 out-of-hospital deaths from a first AMI in the Netherlands in 2000 (figure 2).

Data analysis
The incidence of patients with a first AMI in 2000 (with 95% confidence interval (95% CI)) was computed by age and gender. This was done by dividing age and gender-specific numbers of patients with a first AMI in 2000 with corresponding age and gender-specific numbers of unique persons in the PR at 1 July 2000. Unique persons were defined as persons who were unique in the population on the combination of values of the linkage variables. In this way, the numbers of unique persons in the PR at 1 July were used as an estimate of person-years at risk. The incidence in men was compared with the incidence in women by calculating incidence rate ratios (or relative risks) (with 95% CI) by age. The 95% confidence intervals were estimated assuming that the observed number of AMI cases followed a Poisson distribution. The proportion of out-of-hospital deaths of the total number of first AMI
The incidence of a first AMI increased with age in both men and women (Table 3). In men, the incidence (per 100,000 persons per year) increased from 2 (95% CI 2 to 3) in the age group younger than 30 years to 2996 (95% CI 2718 to 3274) in the age group of 90 years and older, in
Table 2. Characteristics of out-of-hospital deaths from a first acute myocardial infarction in the Netherlands in 2000

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men</th>
<th>Women</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>6972</td>
<td>5747</td>
<td>12,719</td>
</tr>
<tr>
<td>Age at death (years):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Mean (SD)</td>
<td>72.0 (11.2)</td>
<td>80.0 (11.5)</td>
<td>75.6 (11.1)</td>
</tr>
<tr>
<td>• Median</td>
<td>74.0</td>
<td>82.1</td>
<td>77.9</td>
</tr>
<tr>
<td>Origin (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Native</td>
<td>90.4</td>
<td>89.7</td>
<td>90.1</td>
</tr>
<tr>
<td>• Non-native</td>
<td>9.6</td>
<td>10.3</td>
<td>9.9</td>
</tr>
<tr>
<td>Primary cause of death (ICD-10 code) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Acute myocardial infarction (I21)</td>
<td>73.6</td>
<td>70.5</td>
<td>72.1</td>
</tr>
<tr>
<td>• Subsequent myocardial infarction (I22)</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>• Current complications following acute myocardial infarction (I23)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>• Other forms of acute ischaemic heart disease (I24.8)</td>
<td>0.2</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>• Unspecified acute ischaemic heart disease (I24.9)</td>
<td>1.0</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>• Cardiac arrest (I46)</td>
<td>20.2</td>
<td>21.3</td>
<td>20.7</td>
</tr>
<tr>
<td>• Sudden death with unknown cause (R96)</td>
<td>4.9</td>
<td>6.4</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Table 3. Incidence (per 100,000 persons per year) of first acute myocardial infarction (AMI) by age and gender in the Netherlands in 2000

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Hospitalised patients</th>
<th>Out-of-hospital deaths</th>
<th>Total number of first AMI cases</th>
<th>Total number of persons*</th>
<th>Incidence</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>37</td>
<td>21</td>
<td>58</td>
<td>2,576,315</td>
<td>2</td>
<td>2-3</td>
</tr>
<tr>
<td>30-39</td>
<td>356</td>
<td>98</td>
<td>454</td>
<td>1,098,227</td>
<td>41</td>
<td>38-45</td>
</tr>
<tr>
<td>40-49</td>
<td>1450</td>
<td>346</td>
<td>1796</td>
<td>1,011,713</td>
<td>178</td>
<td>169-186</td>
</tr>
<tr>
<td>50-59</td>
<td>2960</td>
<td>845</td>
<td>3805</td>
<td>892,870</td>
<td>426</td>
<td>413-440</td>
</tr>
<tr>
<td>60-69</td>
<td>3344</td>
<td>1375</td>
<td>4719</td>
<td>606,735</td>
<td>778</td>
<td>756-800</td>
</tr>
<tr>
<td>70-79</td>
<td>3291</td>
<td>2189</td>
<td>5480</td>
<td>399,574</td>
<td>1371</td>
<td>1335-1408</td>
</tr>
<tr>
<td>80-89</td>
<td>1276</td>
<td>1721</td>
<td>2997</td>
<td>138,020</td>
<td>2171</td>
<td>2094-2249</td>
</tr>
<tr>
<td>≥90</td>
<td>69</td>
<td>377</td>
<td>446</td>
<td>14,886</td>
<td>2996</td>
<td>2718-3274</td>
</tr>
<tr>
<td>All ages</td>
<td>12,783</td>
<td>6972</td>
<td>19,755</td>
<td>6,738,340</td>
<td>293</td>
<td>289-297</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>13</td>
<td>8</td>
<td>21</td>
<td>2,481,992</td>
<td>1</td>
<td>1-1</td>
</tr>
<tr>
<td>30-39</td>
<td>90</td>
<td>41</td>
<td>131</td>
<td>1,067,573</td>
<td>12</td>
<td>10-14</td>
</tr>
<tr>
<td>40-49</td>
<td>374</td>
<td>102</td>
<td>476</td>
<td>989,198</td>
<td>48</td>
<td>44-52</td>
</tr>
<tr>
<td>50-59</td>
<td>692</td>
<td>186</td>
<td>878</td>
<td>867,887</td>
<td>101</td>
<td>95-108</td>
</tr>
<tr>
<td>60-69</td>
<td>1250</td>
<td>530</td>
<td>1800</td>
<td>637,225</td>
<td>282</td>
<td>269-295</td>
</tr>
<tr>
<td>70-79</td>
<td>2108</td>
<td>1536</td>
<td>3644</td>
<td>531,798</td>
<td>685</td>
<td>663-708</td>
</tr>
<tr>
<td>80-89</td>
<td>1333</td>
<td>2326</td>
<td>3859</td>
<td>283,866</td>
<td>1399</td>
<td>1377-1402</td>
</tr>
<tr>
<td>≥90</td>
<td>215</td>
<td>998</td>
<td>1213</td>
<td>54,302</td>
<td>2226</td>
<td>2100-2351</td>
</tr>
<tr>
<td>All ages</td>
<td>6275</td>
<td>5747</td>
<td>12,022</td>
<td>6,918,201</td>
<td>174</td>
<td>171-177</td>
</tr>
</tbody>
</table>

*Number of unique persons in the PR on 1 July 2000.
Strengths of our study are the high linkage percentages obtained using this approach, the large size of the cohorts and the lack of selection bias. Recently, a high validity of both the HDR and the PR has been demonstrated. In a random sample of the HDR, 99% of the personal, admission and discharge data and 84% of the principal diagnoses (validated through medical record review by medical specialists) were correctly registered. This unfortunately was based on the principal diagnosis for all patients and all specialties. Therefore subjects with an AMI during hospitalisation but not coded as such may still have been missed, and patients may have been labelled as an AMI, whereas in truth this was not the case. The magnitude of both aspects cannot be estimated.

In a random sample of the PR, over 97% of the addresses were correctly registered and only 0.4% of days and months of birth were missing. Furthermore, over 97% of the uniquely linked hospital admissions resulting from linkage of the HDR with the PR were shown to be correctly linked and the estimated rate of mismatches (false-positive linkages) was approximately 1%. There are a number of critical aspects of our study that need consideration in order to appreciate the findings. First, the information on previous admissions was limited to a maximal five years for the patients (as the numeric part of the postal code has been registered in the hospital register since 1991). Therefore, it seems likely that some ‘first’ AMI patients were actually recurrent AMI patients. It has been estimated that most recurrent events (95%) occur within five years, which means that our incidence rates reflect a 5% overestimate of first-ever AMI. Secondly, the cause of death information used in our study was not validated by medical records or autopsy reports. It is known that the quality of routine mortality statistics varies over time and between countries. Several studies published in the 1980s have shown that the validity of the Dutch national cause of death statistics was higher than the average validity of eight countries of the European Community. More recent studies estimating the degree of misclassification of coronary heart disease are, however, not available. As a consequence, the degree of misclassification in our estimates of the incidence of non-hospitalised first AMI in the Netherlands is unquantifiable, but, as in almost every study using data from vital statistics, some degree of misclassification is inevitable, especially in the very old in whom only limited diagnostic effort is made. Thirdly, when we restrict our out-of-hospital deaths to AMI only, the overall incidence of out-of-hospital death will be reduced by 27%, reducing the overall out-of-hospital death considerably to 28% in men and 39% in women. Fourthly, we assumed that AMI is such a severe and alarming disease that you either die of or are treated in hospital. Therefore, most diagnosed

Table 4. Age-specific gender ratios (RR) of the incidence of first acute myocardial infarction (AMI) in the Netherlands in 2000

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Hospitalised first AMI patients</th>
<th>Out-of-hospital deaths from a first AMI</th>
<th>Total number of first AMI patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR Men/women 95% CI</td>
<td>RR Men/women 95% CI</td>
<td>RR Men/women 95% CI</td>
</tr>
<tr>
<td>&lt;30</td>
<td>2.75 1.46-4.17</td>
<td>2.53 1.12-5.72</td>
<td>2.67 1.62-4.39</td>
</tr>
<tr>
<td>30-39</td>
<td>3.85 3.05-4.85</td>
<td>2.32 1.41-3.53</td>
<td>3.37 2.77-4.09</td>
</tr>
<tr>
<td>40-49</td>
<td>3.79 3.38-4.25</td>
<td>3.32 2.66-4.14</td>
<td>3.69 3.31-4.08</td>
</tr>
<tr>
<td>50-59</td>
<td>4.16 3.83-4.51</td>
<td>4.41 3.77-5.17</td>
<td>4.21 3.91-4.53</td>
</tr>
<tr>
<td>60-69</td>
<td>2.81 2.62-3.00</td>
<td>2.63 2.28-2.90</td>
<td>2.76 2.61-2.91</td>
</tr>
<tr>
<td>70-79</td>
<td>2.08 1.97-2.19</td>
<td>1.90 1.78-2.02</td>
<td>2.00 1.92-2.09</td>
</tr>
<tr>
<td>80-89</td>
<td>1.71 1.59-1.84</td>
<td>1.52 1.43-1.62</td>
<td>1.60 1.52-1.68</td>
</tr>
<tr>
<td>≥90</td>
<td>1.18 0.90-1.54</td>
<td>1.38 1.23-1.56</td>
<td>1.35 1.21-1.50</td>
</tr>
</tbody>
</table>

Figure 3. Proportion of out-of-hospital deaths from a first acute myocardial infarction (AMI) of total number of first AMI patients in 2000 in the Netherlands (error bars represent 95% confidence intervals)
cases of AMI in a population can be identified through combining information on hospital admissions and deaths from national registries as done in our study. Non-fatal and non-hospitalised AMIs were lacking in our estimates. However, unpublished data from the Rotterdam Study, a population-based cohort study among 7,583 men and women aged 55 years and over showed that 17% of all non-fatal AMIs were not hospitalised (personal communication Dr J.C.M. Witteman). Although the Rotterdam Study included data obtained from residential care homes, no information was obtained from nursing homes. Therefore the 1.7% might be an underestimation. Yet, less than 1% of the Dutch population was admitted to a nursing home in 2000 and one may also question the correctness of the diagnosis in those subjects. Unnoticed or silent AMIs were not included in our study, in line with other record linkage studies. If we had included silent AMIs, this probably would have yielded much higher estimates, as De Bruyne et al. demonstrated that the prevalence of silent AMI was only slightly smaller than the prevalence of symptomatic AMI (4.1 and 3.9%) in persons aged 55 years or older. Fifthly, another aspect that needs to be addressed is the small percentage of persons aged 55 years or older. The male-to-female ratio decreased from 4.05 at age 45-54 years to 1.71 at age 75-84 years. Our finding that a substantial proportion of patients with a first AMI died out of hospital is in agreement with data from several other studies. Greenlee et al. reported that about 20% of first AMIs in a general population in the USA from 1992 to 1998 were detected only on death certificates. In another American study, the proportion of out-of-hospital deaths (both first and recurrent events) was estimated at 26% in 1996. In a study among the Jewish population of Jerusalem, 20% of men and 26% of women with a first AMI between 1995 and 1997 died out of hospital. In a Scottish population-based record linkage study, 41% of the patients with a first AMI between 1986 and 1995 did not survive to be admitted to hospital. The risk of out-of-hospital death from a first AMI increased with age from 20% of all first AMI events (deaths plus hospital admissions) in persons <55 years to 62% in persons >85 years. These estimates are comparable with those in our study. In the FINAMI study, the proportion of out-of-hospital deaths of all coronary heart disease deaths was much higher and declined with age. From 1983 to 1997, the proportion of out-of-hospital deaths was 73% in men and 60% in women aged 35-64 years. The proportion of out-of-hospital deaths ranged from 75% in men aged 35-54 years to 41% in men aged 85 years and over. Corresponding figures for women were 65 and 35%.

It seems that there has been no decline in the proportion of out-of-hospital deaths in the Netherlands, since Fracheboud has estimated that one third of patients with a suspected or confirmed AMI died out of hospital in 1985. The large proportion of out-of-hospital deaths from a first AMI shown in our study reinforces the importance of improvements in primary prevention of AMI. Especially in patients who suddenly die before a medical doctor or an ambulance has arrived, treatment options are limited and mortality reduction can be achieved mainly by primary prevention. Furthermore, it is important to minimise the delay to initiation of treatment in patients with out-of-hospital cardiac arrest, as a shorter delay is shown to be associated with improved survival. In conclusion, our study provides, for the first time, incidence estimates of first AMI based upon virtually the entire Dutch population. Expected differences in incidence with regard to age and gender were shown. The large proportion of out-of-hospital deaths reinforces the importance of primary prevention of AMI.

ACKNOWLEDGEMENTS

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Failure of CHOP with rituximab for lymphomatoid granulomatosis

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ABSTRACT

We present a 66-year-old male patient with pulmonary lymphomatoid granulomatosis. The patient had progressive disease after three courses of CHOP and rituximab and, therefore, treatment with interferon-α2b 5 x 10^6 IE three times a week was started. This resulted in stable disease for five months. Subsequently, progression occurred and the patient died 12 months after initial presentation. Lymphomatoid granulomatosis is a rare, poor-risk, Epstein-Barr virus related, B cell lymphoproliferative disease. There is no standard treatment but promising results have been reported with rituximab, either as monotherapy or in combination with chemotherapy. This case demonstrates that lymphomatoid granulomatosis is still a chemotherapy-resistant disease in some patients despite addition of rituximab. A review of the literature regarding aetiology, clinical features, diagnosis and treatment options is presented.

KEYWORDS

Epstein-Barr virus, interferon, lymphomatoid granulomatosis, lymphoproliferative disease, rituximab

CASE REPORT

A 66-year-old man presented with superficial thrombophlebitis of his left leg. A routine chest X-ray showed multiple round nodules, predominantly in the lower lung fields (figure 1). The patient had a history of diabetes, hypertension, hypercholesterolaemia, claudication and a temporary paralysis of the facial nerve. He had lost 10 kg of weight in the previous six months and suffered from night sweats. During analysis he developed chills and fever with a productive cough and shortness of breath. On lung auscultation he had crackles and rales on both sides and diminished breath sounds over the lower left lung. There was no evidence of lymphadenopathy, hepatomegaly or splenomegaly. Laboratory tests showed a high sedimentation rate, a microcytic anaemia, a mild leucocytosis and thrombocytosis and an elevated alkaline phosphatase and γ-glutamyl transferase (table 1). A transbronchial lung biopsy was inconclusive and an open lung biopsy was performed. A small wedge resection containing a 3.5 cm white mass was removed. Histology showed necrotic tissue surrounded by a polymorphous infiltrate of lymphoid cells. Large CD20 positive B cells were present in a background of small CD3 positive T cells. This infiltrate was concentrated...
No yeast, fungi or acid-fast bacilli were detected but in situ hybridisation for Epstein-Barr virus (EBV) was strongly positive (figure 2). The diagnosis of lymphomatoid granulomatosis grade III was made. We treated him with intensified CHOP and rituximab every two weeks (table 2). After the third cycle a CT scan of the chest showed progressive disease (figure 3). The number as well as the size of the lesions had increased. Our patient was subsequently treated with interferon-α2b. Due to side effects, the maximum tolerated dose was 5 million IE three times a week. On this regimen he was stable for five months, but died 12 months after initial presentation due to progressive disease.

**Table 1: Laboratory results**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR</td>
<td>&gt;120 mm/h</td>
<td>Base excess</td>
<td>-0.7 mmol/l</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>5.9 mmol/l</td>
<td>PO₂</td>
<td>7.9 kPa</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>0.29 l/l</td>
<td>Urea</td>
<td>9.9 mmol/l</td>
</tr>
<tr>
<td>MCV</td>
<td>78 fl</td>
<td>Creatinine</td>
<td>81 μmol/l</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>11.6 x 10⁹/l</td>
<td>Sodium</td>
<td>134 mmol/l</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.25 x 10⁹/l</td>
<td>Potassium</td>
<td>4.0 mmol/l</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.07 x 10⁹/l</td>
<td>AP</td>
<td>193 U/l</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>9.58 x 10⁹/l</td>
<td>γGT</td>
<td>234 U/l</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.5 x 10⁹/l</td>
<td>ASAT</td>
<td>39 U/l</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1.1 x 10⁸/l</td>
<td>ALAT</td>
<td>23 U/l</td>
</tr>
<tr>
<td>Platelets</td>
<td>415 x 10⁹/l</td>
<td>LDH</td>
<td>305 U/l</td>
</tr>
<tr>
<td>pCO₂</td>
<td>4.3 kPa</td>
<td>Bilirubin</td>
<td>&lt;5 μmol/l</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>22.4 mmol/l</td>
<td>ANCA</td>
<td>Negative</td>
</tr>
</tbody>
</table>

ESR = erythrocyte sedimentation rate; MCV = mean corpuscular volume; AP = alkaline phosphatase; γGT = gamma glutamyltransferase; ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase; LDH = lactate dehydrogenase; ANCA = antineutrophil cytoplasmic antibodies.

**Table 2: Chemotherapy regimen**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Route</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide</td>
<td>750 mg/m²</td>
<td>IV</td>
<td>Day 1</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>50 mg/m²</td>
<td>IV</td>
<td>Day 1</td>
</tr>
<tr>
<td>Vincristine</td>
<td>1.4 mg/m²</td>
<td>IV</td>
<td>Day 1</td>
</tr>
<tr>
<td>Prednisone</td>
<td>100 mg</td>
<td>PO</td>
<td>Day 1-5</td>
</tr>
<tr>
<td>Rituximab</td>
<td>375 mg/m²</td>
<td>IV</td>
<td>Day 3 (cycle 1-2), day 1 (cycle 3-6)</td>
</tr>
<tr>
<td>G-CSF (pegfilgrastim)</td>
<td>6 mg</td>
<td>SC</td>
<td>Day 2</td>
</tr>
</tbody>
</table>

G-CSF = granulocyte-colony stimulating factor; IV = intravenous; PO = per os; SC = subcutaneous.

**Figure 2.** (A) Polymorphic tumour cells in a background of small lymphocytes (HaE); (B) CD20 immunostain with a membranous positivity of large tumour cells; (C) most nuclei of the tumour cells are stained by EBER (EBV RNA in situ hybridisation)

Original magnification: 40X. Courtesy of Dr James E Boers, Isala Clinics, Zwolle.

**Figure 3.** CT scan of the chest (A) before start of treatment; (B) after three cycles CHOP with rituximab

**Introduction**

Lymphomatoid granulomatosis is a rare lymphoproliferative disease. It was first described in 1972 by Liebow et al. during their studies of patients with Wegener's granulomatosis. Lymphomatoid granulomatosis was...
described as an atypical angiocentric and angiodestructive lymphoproliferative disease, predominantly located in the lungs but sometimes present at other extranodal sites. After this first description there has been a lot of controversy regarding the concept and the nature of lymphomatoid granulomatosis. An overview of the literature about lymphomatoid granulomatosis, clinical and histological features and effectiveness of different treatment modalities is presented.

**HISTORY OF THE CONCEPT**

It has long been recognised that immunocompromised patients are predisposed to develop lymphomatoid granulomatosis. The disease has been reported in patients with primary immunodeficiency as well as in patients with secondary immunodeficiency. Furthermore, Sordillo et al. found that four of five lymphomatoid granulomatosis patients were unresponsive to common skin test antigens and that the fifth patient showed partial anergy. Fauci et al. reported that three out of six lymphomatoid granulomatosis patients were anergic in response to common skin tests. Due to clinical and histological similarities, it was suggested that lymphomatoid granulomatosis and polymorphous reticulosis (nasal and nasal type natural killer (NK) T cell lymphoma) were part of the same disease. Together these diseases were called angiocentric immunoproliferative lesions (AIL). A staging system for AIL, based on histological characteristics, was developed and proved to have prognostic value in small series of patients. Nichols et al. postulated that lymphomatoid granulomatosis was a T-cell lymphoma because the majority of the lymphocytes consisted of T cells and this was the leading opinion for more than a decade. Pisani et al. suggested that lymphomatoid granulomatosis was not a clinicopathological entity but a histological response to different stimuli, such as haematological malignancies, solid tumours, viral infections and autoimmunity. This was, however, not a widely held opinion. In their initial description Liebow et al. suggested a relationship between lymphomatoid granulomatosis and EBV infection. This relationship was confirmed in 1990 when EBV DNA was found in tissue samples of 21 out of 29 patients with lymphomatoid granulomatosis. Guinee et al. combined in situ hybridisation for EBV with immunohistochemistry in tissue samples from ten patients with lymphomatoid granulomatosis. In each case EBV was only present in the B cells. In six out of nine patients tested, immunoglobulin heavy chain rearrangement showed a monoclonal pattern. Wilson et al. confirmed that the EBV expression was restricted to B cells, in four patients. In analogy to post-transplant lymphoproliferative disease (PTLD), they also demonstrated two B cell clones in one patient and three B cell clones in another.

These findings led to the current opinion that lymphomatoid granulomatosis is an EBV-associated B cell lymphoproliferative disease. The majority of the infiltrating cells are reactive T lymphocytes recruited in response to EBV infection. Cellular immunodeficiency probably prohibits EBV elimination in the majority of patients. In the WHO classification system lymphomatoid granulomatosis is grouped together with PTLD as ‘B cell lymphoproliferative disorders with uncertain malignant potential’.

**CLINICAL FEATURES AND HISTOPATHOLOGY**

Two large and three smaller series of patients with lymphomatoid granulomatosis have been described. The largest series consists of 152 cases that were identified in Liebow’s consultation files. Lymphomatoid granulomatosis has been diagnosed in patients from 4 to 85 years of age, but generally patients are between 40 and 60 years of age. Men are more frequently affected than women with male: female ratios ranging from 2:1 to 3:1. Most patients present with pulmonary symptoms such as cough, shortness of breath or chest pain and the majority of patients have systemic symptoms such as weight loss, fever and night sweats. Of the patients, 20 to 40% develop skin manifestations, either an erythematosus rash or, less frequently, skin nodules. Almost a third of the patients develop neurological symptoms such as confusion, ataxia, hemiparesis or seizures, mostly due to mass lesions in the central nervous system (CNS). Cranial nerve palsies and peripheral polyneuropathy have also been described. The disease is typically located in the lungs. Localisation in the liver and kidneys occurs in approximately one third of patients but is generally asymptomatic. Hepatomegaly and splenomegaly are present in less than 20% of the patients and lymphadenopathy is even less common at presentation (7-8%). Pisani et al. detected bone marrow localisation in one of 19 patients and Fauci et al. in five of 15 patients. Bone marrow investigation was not described in the two largest series.

Laboratory investigation shows nonspecific abnormalities at initial presentation. Erythrocyte sedimentation rate is either normal or elevated. White cell count is normal in 50%, elevated in 30% and decreased in 20% of patients. Mild anaemia is sometimes present and during disease progression, pancytopenia caused by the haemophagocytic syndrome occasionally develops. The majority of the patients have atypical abnormalities in immunoglobulin concentrations and about a third have mild elevations in liver enzyme levels. Chest X-rays show bilateral lesions in 71 to 92% of patients. Multiple nodules are most frequently seen while diffuse,
reticular or nodular infiltrates are less often described. Rarely, lymphadenopathy, pleural effusions, cavitations or solitary masses are present. Mortality of patients with lymphomatoid granulomatosis ranges from 38% to 65% in the different studies. In patients who die from their disease, the median survival is 11.3 months and death is generally caused by massive pulmonary destruction. Older studies suggest that leucopenia, fever, anergy in reaction to common skin test antigens, young age and localisation in the CNS are poor prognostic signs. Diagnosis should be made on a dominant noncutaneous lesion. Transbronchial biopsy is not recommended since it is diagnostic in only 27% of cases while open lung biopsy specimens are uniformly positive. Histology typically shows a polymorphous infiltrate predominantly consisting of lymphocytes although plasma cells, histiocytes and immunoblasts can also be present. The majority of the lymphocytes are T cells and CD4 positive as well as CD8 positive subsets, without malignant features, are present. Immunoblasts are large atypical CD20 positive B cells. Populations of B cells are either monoclonal, oligoclonal or polyclonal and most B cells contain EBV DNA. The infiltrate is concentrated around small arteries and veins and causes destruction of the vessels. Necrosis develops due to direct T cell invasion, causing infarction, and due to destruction of the vessels resulting in fibrinoid necrosis. The latter may be mediated by EBV latent membrane protein which can cause upregulation of both IP-10 (interferon-γ inducible protein) and Mig (monokine induced by interferon-γ), which have been shown to cause endothelial and vascular damage.

Skin lesions often lack EBV-positive B cells and resemble vasculitis. In 1979, it was already suggested that higher numbers of atypical lymphoreticular cells are associated with poor outcome. Guineee et al. demonstrated a negative correlation between the amount of EBV-positive B cells and survival. They suggested a grading system in which grade I lesions contain few, if any, EBV-positive B cells, grade II lesions show more EBV-positive B cells but less than 100 per high power field and grade III lesions consist of infiltrates with more than 100 EBV-positive B cells per high-power field. In patients at risk for PTLD, serial quantitative polymerase chain reaction (PCR) analyses of EBV DNA in plasma has been shown to have predictive value for development of PTLD although there is considerable overlap between patients with symptomatic EBV reactivation without PTLD and patients with PTLD. Response to treatment in patients with PTLD is accompanied by a prompt decline in viral copy number. To our knowledge there are no data available about the value of determination of EBV load for diagnosis and guidance of therapy for patients with lymphomatoid granulomatosis. It is tempting, however, to hypothesise that serial measurements can be used to evaluate response to treatment.

**TREATMENT**

Because lymphomatoid granulomatosis is a rare disease, very few treatment studies have been conducted and there is no standard treatment. Depending on severity at presentation most patients are treated with corticosteroids, either as single agent or combined with cyclophosphamide, or with other chemotherapeutic agents as CHOP or COP regimens. Radiotherapy has been used for CNS and orbital localisations. The largest series described is a retrospective analysis of different treatment strategies in 147 patients. Patients were classified according to treatment as follows: group I: corticosteroids (n=67), group II: corticosteroids combined with chemotherapy (n=42), group III: chemotherapy (n=15), group IV: antibiotics or no treatment (n=21), and group V: miscellaneous (n=4). Mortality varied from 64 to 69% and durable complete remission ranged from 24 to 27%. No significant differences were found between the groups.

Fauci et al. treated 15 patients prospectively with cyclophosphamide (2 mg/kg/day orally) and prednisone (1 mg/kg/day orally). This protocol was based on treatment regimens for Wegener's granulomatosis. Two patients only received prednisone and died of progressive disease before diagnosis was clear. Seven patients achieved complete remission and remained disease free after a median follow-up of 5.2 years. Six patients treated with prednisone and cyclophosphamide died of progressive disease. Three of these six patients received combination chemotherapy without success.

Raez et al. treated a 51-year-old patient with lymphomatoid granulomatosis with ProMACE-MOPP, a multiagent chemotherapeutic regimen for aggressive lymphomas. The patient responded but disease recurred one month after completion of six cycles of the chemotherapy. The patient subsequently received cyclosporin-A and achieved complete remission within eight weeks. After discontinuing maintenance therapy two years later, disease recurred within three weeks. A third remission was achieved after restarting cyclosporin-A and the patient remained in remission for a follow-up of four years after diagnosis.

Wilson et al. treated four patients with interferon-α2b which has antiviral, antiproliferative and/or immunomodulatory effects, based on the assumption that lymphomatoid granulomatosis is related to PTLD. Three patients received interferon as first-line treatment and one patient received interferon after an early relapse on six cycles of CHOP chemotherapy. All four patients had responded by three months, three were in complete remission and remained disease free after 36 to 60 months of follow-up. One patient died after discontinuation of treatment.

The same investigators set up a phase II study with dose-adjusted interferon-α for patients with lymphomatoid granulomatosis grades I and II and EPOCH chemotherapy.
(infusional etoposide, vincristine, doxorubicin, with bolus cyclophosphamide and oral prednisone) for lymphomatoid granulomatosis grade III. Interferon is started at 5-10 x 10^6 IE three times a week and the dose is escalated until disease regression or tolerance is achieved. Accrual is still ongoing and preliminary results were published in 1999. Of twelve evaluable patients on interferon, eight were in remission for a median duration of 19 months, four rapidly progressed to grade III lymphomatoid granulomatosis. Five evaluable patients were treated with chemotherapy, three achieved complete remission, two partial remission. Three of these five patients developed lower grade lymphomatoid granulomatosis and were subsequently treated with interferon, in two with good results. For the third patient follow-up was too short for evaluation. Two further cases of lymphomatoid granulomatosis were reported for first-line treatment with interferon. One patient relapsed on discontinuation after three months; the other patient was treated for 18 months with a good result. In 1986 Bernstein et al. described a 19-year-old patient with lymphomatoid granulomatosis with recurrent disease after COP chemotherapy. The patient received a bone marrow transplant from his HLA-compatible brother and remained in remission during a follow-up of more than three years. To our knowledge no patients have been reported for treatment with nonmyeloablative allogeneic stem cell transplantation. This might be an interesting treatment option for restoring the presumed underlying immunocompromised status while reducing toxicity compared with myeloablative allogeneic stem cell transplantation.

In two case reports, successful treatment of lymphomatoid granulomatosis with autologous stem cell transplantation has been described after failure of combination chemotherapy. The patients were in remission for 12 months and eight years respectively. The last patient received maintenance therapy with interferon for almost four years. Rituximab has been recognised as a promising treatment option in lymphomatoid granulomatosis over the last few years. Six patients were treated with rituximab monotherapy and three patients had durable complete remission, one patient had a major response after four courses but died of haemoptysis and two patients had progressive disease. Two patients with lymphomatoid granulomatosis treated with CHOP in combination with rituximab have been described. One patient was still in complete remission after 18 months of follow-up. The other patient concomitantly received systemic and intrathecal methotrexate for CNS localisation. He had a partial response of pulmonary lesions and stable CNS lesions two months after starting therapy; however, CNS lesions were progressive after six months. He then received radiation therapy and four courses of rituximab monotherapy with partial response of CNS lesions.

DISCUSSION

This is the first description of failure of the combination of CHOP chemotherapy with rituximab to induce a response in lymphomatoid granulomatosis. Many patients with lymphomatoid granulomatosis have been treated with CHOP chemotherapy but data on efficacy are lacking. Nevertheless, CHOP was the recommended treatment for patients with aggressive grade I and II disease and for all patients with grade III disease before rituximab became available. Anti-CD20 immunotherapy is a rational treatment option for several reasons. Firstly, the neoplastic cell population in lymphomatoid granulomatosis consists of CD20 positive B cells. Secondly, the addition of rituximab to CHOP chemotherapy for diffuse large B cell lymphoma has been shown to improve response rate, progression free and overall survival. Furthermore, rituximab is an important drug for treating PTLD, a disease closely related to lymphomatoid granulomatosis.

In two earlier reports a complete and a partial response with CHOP with rituximab for lymphomatoid granulomatosis were described. Our patient, however, had progressive disease on three treatment cycles. This case shows that lymphomatoid granulomatosis is still a chemotherapy-resistant disease in some patients despite the addition of rituximab. Since promising results of interferon for lymphomatoid granulomatosis have been described in a limited number of patients we treated our patient with interferon. He was stable for five months on interferon 5 x 10^6 IE, three times a week; however, he finally succumbed to progressive disease 12 months after initial presentation. Stable disease during interferon treatment in our patient should be considered a response because the patient had rapidly progressive disease before starting treatment. Unfortunately we were not able to increase the dose because of side effects. Otherwise an objective response might have been possible, as in one of the patients described by Wilson et al. who had complete remission after gradual dose increases of interferon up to 40 x 10^6 IE three times a week. Haematopoietic stem cell transplantation has successfully been used in refractory lymphomatoid granulomatosis. We did not consider our patient to be a candidate for transplantation because of infectious problems, substantial comorbidity and poor condition.

NOTE

This case was presented at the Autumn Conference of the Netherlands Society of Haematology, (NVvH) in Lunteren on 4 November 2004.

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Oosting-Lenstra, et al. R-CHOP for lymphomatoid granulomatosis. D E C E M B E R 2 0 0 7 , V O L . 6 5 , N O . 1 1
Watery diarrhoea: an unusual manifestation of breast cancer

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Departments of 1Internal Medicine, 2Gastroenterology and 3Pathology, Renier de Graaf Gasthuis, Delft, the Netherlands, *corresponding author

ABSTRACT

Analysis of an 83-year-old male presenting with diarrhoea showed secretory diarrhoea. Serum levels of gastrin and pancreatic polypeptide were elevated. Somatostatin-receptor scintigraphy revealed a hot spot in the left thoracic wall and subsequently, breast adenocarcinoma with neuroendocrine differentiation was diagnosed. Postoperatively, the patient made an uneventful recovery. The relationship between the clinical picture, the results of pathological examination and hormonal analysis is discussed and put into perspective.

KEYWORDS

Breast cancer, gastrin, neuroendocrine tumour, pancreatic polypeptide, watery diarrhoea

INTRODUCTION

Several neoplastic disorders can cause chronic watery diarrhoea attributable to hormonal-mediated response. These include pancreatic endocrine tumours, carcinoid syndromes and medullary thyroid cancer. These disorders are not usually considered to be part of the differential diagnosis of chronic diarrhoea because of their rarity among all other causes of diarrhoea. We describe a patient with severe watery diarrhoea for whom a neuroendocrine tumour of the breast was the most probable explanation. To the best of our knowledge, this association has not been described before.

CASE REPORT

An 83-year-old man presented with a three-week history of progressive diarrhoea. Apart from gastric outlet obstruction due to peptic ulcer disease, treated with esomeprazole 40 mg daily, his medical history was unremarkable. The diarrhoea was massive and watery (up to three litres daily). He had a weight loss of 5 kg. Treatment with loperamide and ciprofloxacin gave no relief.

Physical examination revealed no abnormalities apart from slight dehydration. The results of laboratory examination are shown in table 1. The patient was treated with parenteral fluids and supplementation of potassium. Stool examination for bacterial pathogens, parasites and toxins showed no pathogenic micro-organisms. Biochemical analysis of the stools showed elevated sodium and potassium excretion: sodium 68 mmol/l (normal <10 mmol/l), potassium 62 mmol/l (normal 5-15 mmol/l). The calculated osmotic gap (290- 2x (Na+K)) was 30 mOsmol/kg, suggestive of secretory diarrhoea. Upper gastrointestinal endoscopy showed gastric retention due to...
pyloric stenosis, but no signs of active ulcer disease and the mucosa of the stomach appeared normal. Colonoscopy was normal. Determination of serum peptides showed elevated levels of gastrin and pancreatic polypeptide (PP), 1290 ng/l (normal <150) and 197 pmol/l (normal <100), respectively, while vasoactive intestinal polypeptide (VIP) measured <5 ng/l (normal <20). The elevated levels of gastrin and PP in the serum were suggestive of a neuroendocrine tumour. Treatment with octreotide established a relief in the severity of the diarrhoea. Computed tomography (CT) scan of the abdomen revealed no abnormalities. Somatostatin-receptor scintigraphy with indium-labelled octreotide showed an increased uptake in the left thoracic wall. Combining the data of the scintigraphy and CT scan confirmed the localisation of a tumour mass in the left breast (figure 1). Subsequent mammography revealed an irregular lump just behind the nipple. An ultrasound-guided biopsy of the left breast mass was performed. Cytological examination confirmed an adenocarcinoma in the left breast. The patient underwent mastectomy and axillary lymph node dissection. Pathological examination showed a ductal adenocarcinoma. Immunohistochemical phenotyping of the tumour confirmed the diagnosis of an adenocarcinoma with neuroendocrine differentiation (figure 2 A-D). Postoperatively, the diarrhoea disappeared.
and the patient had an uneventful recovery. As an outpatient he remained asymptomatic during a follow-up of 12 months. Hormonal analysis, eight weeks after surgery, showed a normal level of PP (62 pmol/l). Serum gastrin level remained high at 997 ng/l.

DISCUSSION

Feyrter and Hartmann were the first to describe two patients with breast cancers with carcinoid growth patterns. They stated that neuroendocrine differentiation can be identified in up to 30% of breast cancers. Neuron-specific enolase (NSE) is well-known marker to demonstrate neuroendocrine differentiation and the same is true for chromogranin and synaptophysin. However, NSE-positive breast tumours are not always immunoreactive for peptide hormones and usually, neuropeptide immunostaining is only found in single cells or small groups of cells (most frequently for gastrin and PP). The clinical meaning of a hormonal content is unknown, possibly related to local growth regulation and only very rarely associated with clinical signs and symptoms (known for norepinephrine and adrenocorticotropin). There is no consensus with respect to the definition of neuroendocrine differentiated breast cancer. Some investigators consider tumours with even a minimal population of neuroendocrine cells (1-2%) to be neuroendocrine tumours, while others only classify a tumour as neuroendocrine when the majority of tumour cells display neuroendocrine characteristics. The present report supports the diarrhoeogenic potentials of neuroendocrine cells originating from a malignancy outside the gut or pancreas, more specifically from a male breast cancer.

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REFERENCES


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Synopsis of the Dutch multidisciplinary guideline for the diagnosis and treatment of hereditary haemochromatosis

D.W. Swinkels¹*, A.T.M. Jorna², R.A.P. Raymakers³, on behalf of the members of the working party

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Abstract

Hereditary haemochromatosis (HH) is a disease related to mutations in the HFE gene and can lead to progressive iron accumulation, especially in the liver, eventually resulting in organ damage. We have developed guidelines for the diagnosis and treatment of this disease according to CBO methodology (Dutch Institute for Healthcare Quality). The prevalence of clinical symptoms such as fatigue, arthropathies, impotence and diabetes mellitus among homozygotes was similar to that in a control population. Nevertheless, we recommend the assessment of serum iron indices when these symptoms remain unexplained. When transferrin saturation is >45% and ferritin exceeds local reference ranges, HFE mutations should be investigated. Homozygosity for the C282Y mutation or combined C282Y/H63D mutation confirms the diagnosis of HFE-related HH. Liver biopsy is recommended when ferritin exceeds 1000 µg/l to establish the presence or absence of cirrhosis, which will affect prognosis and management. Iron accumulation confirmed by magnetic resonance imaging (MRI) in the absence of the homozygous C282Y mutation or the combined C282Y/H63D genotype may justify a search for rare hereditary forms of non-HFE HH in a specialised centre. The literature supports the benefits of adequate phlebotomy and the screening of first-degree relatives of index patients with clinically overt HH. Overall, the guidelines presented here are to a great extent based on the expert opinion of the working party, as the quantity of evidence that met predefined criteria posed by the evidence-based approach was small. We therefore recommend world-wide efforts to collaboratively address these remaining issues.

Methodology

Development method

The working group adopted the CBO (the Dutch Institute for Healthcare Quality, www.cbo.nl) method of evidence-based guideline development for answering a number of predefined clinical questions. A literature search exploiting MESH/(thesaurus) terms and free text in the databases Medline, Embase, and the Cochrane library was performed until mid 2005. Next to literature from systematic searches, additional articles were acquired by bibliographies of key reviews and included studies. Furthermore, relevant studies that appeared later than 2005 were included, as well as (inter)national guidelines.¹⁻⁶

Procedures

The concepts of the chapters of the guideline prepared by individual members of the working party on the basis of the best available evidence were discussed and amended in plenary sessions. Literature was reviewed and evidence was classified according to the CBO rating scheme. The members abstracted studies into evidence tables using condition definitions and diagnostic criteria. If scientific evidence was lacking, issues were discussed until the working party members agreed upon text and recommendations. The draft guideline was sent to the representing professional societies for comments. These comments were discussed by the working group and incorporated in the final version of the guideline. Two years after the first meeting of the working party, the guideline was approved by the boards of the participating scientific associations in May and June 2007 and made available on line along with the evidence tables (in Dutch: http://www.internisten.nl/home/richtlijnen/niv/niv/hemochromatose-niv/nvk)}
Figure 1. Diagnostic diagram for suspected iron accumulation

1. **Determine ferritin and transferrin saturation**
   - Raised ferritin > reference lab for age/sex
   - Ferritin not raised
   - Exclude: infections, inflammations, secondary haemochromatosis

2. **TS > 0.45**
   - DNA diagnostics
   - HFE
   - C282Y/C282Y or C282Y/H63D
   - MRI: Iron too high
   - Diagnostics HH type 1-4
   - Ferritin ≤1000 µg/l
   - Liver biopsy (fibrosis/cirrhosis?)
   - Phlebotomy until ferritin < 50 µg/l
   - Follow-up
   - Family screening

3. **TS ≤ 0.45**
   - HH cannot explain current symptoms
   - MRI: Iron normal
   - No C282Y/C282Y or C282Y/H63D
   - HH cannot explain current symptoms

TS = transferrin saturation; HH = hereditary haemochromatosis; MRI = magnetic resonance imaging.

*Type 1 diagnostics consists of testing the gene for rare mutations (i.e., not the frequent C282Y and H63D mutations).

In addition to the information in the diagram, the diagnostic route taken may depend on:
- Clinical presentation
- Haemoglobin (low in secondary types of iron accumulation and in some forms of ferroportin disease)
- Family history (hereditary disease)
- Concomitant clinical pictures (hepatitis, alcohol abuse)
- Age upon presentation (young in the case of juvenile haemochromatosis)
SUMMARY OF THE GUIDELINE

Epidemiology
Hereditary haemochromatosis (HH) is a disease that is characterised by progressive iron accumulation, especially in the liver, eventually resulting in organ damage. HH is a frequent hereditary condition. In Northern Europe, 0.5 to 1.0% of the population is homozygous for the C282Y mutation and 1 to 3% has the combined C282Y/H63D genotype. However, the relation between genotype and the biochemical and clinical expression (reviewed in references 7-10) remains unclear.

Morbidity
Iron accumulation results in a number of nonspecific symptoms, e.g. general health disturbance, joint problems, diabetes mellitus, fatigue, abdominal symptoms, impotence, cardiovascular diseases and skin pigmentation. However, none of these individual symptoms have been proved to occur more frequently among subjects with the genetic condition of HH than among control subjects. The occurrence of any of these symptoms, therefore, does not justify the performance of diagnostic tests for HH in first-line care. However, in accordance with international guidelines, the working group believes that assessment of the serum iron status should be considered in patients of Northern European descent who have been referred to a specialist after at least six months of unexplained symptoms as described above. The diagnostic diagram in figure 1 outlines the subsequent diagnostic and therapeutic strategies.

Diagnostic strategy
Serum iron indices
During the first diagnostic phase, the combined measurement of serum iron, transferrin (and the calculation of transferrin saturation (TS)) and ferritin, offers a simple and reliable approach for determining the amount of iron in the body. When TS is >45% and ferritin levels exceed the reference laboratory values, HFE mutations should be investigated. However, hyperferritinaemia and raised TS are observed both in HH and in secondary haemosiderosis with anaemia. Conditions with increased TS or ferritin but without significant iron accumulation including infections and inflammations, excessive alcohol use, hepatic disorders and metabolic syndrome should be considered.

Genotypic testing
During the second diagnostic phase, homozygosity for the C282Y mutation or the combined C282Y/H63D genotype confirms an HFE-related form of HH.

Role of liver biopsy and MRI
To diagnose cirrhosis a liver biopsy remains the gold standard and is recommended when serum ferritin is >1000 μg/l. In case of raised serum iron parameters without homozygosity for the C282Y mutation or the combined C282Y/H63D genotype, an MRI can be performed as a semiquantitative assessment of iron in the liver. MRI-confirmed iron accumulation in the absence of the C282Y mutation or the combined C282Y/H63D genotype justifies a search for rare hereditary forms of non-HFE HH in a specialised centre.

FAMILY SCREENING
In the third diagnostic phase, relatives to the first degree must be evaluated on the basis of iron parameters and, in the event of an HFE-related form of HH, on the basis of HFE genotyping as well. An index patient’s siblings and his children/parents have a 25 and 5% chance, respectively, of being predisposed to HH.

TREATMENT
The treatment of haemochromatosis involves phlebotomy, which can prevent and possibly reverse tissue damage. During the depletion phase, weekly 500 ml bloodlettings are performed based on haemoglobin and serum ferritin, until ferritin levels are less than 50 μg/l. During the maintenance phase, ferritin levels are kept within reference values, which may involve several phlebotomies per year.

DISCUSSION
Despite the wealth of information about this disease that has accumulated over the years, diagnostic and therapeutic strategies that are recommended in the various reviews throughout the literature as well in our and other guidelines appear to lack solid evidence and are to a great extent based on expert opinions.

During the development of the guidelines, we identified the following parts in the work-up and treatment of patients that in our opinion urgently need a more solid scientific basis:

- the natural history of the relation between genotype and phenotype in the disease, with respect to sex, age, and genetic and environmental factors;
- determination of the optimal approach to screening for iron overload;
- the level of the serum iron indices above which disease manifestations as fatigue and arthritis are likely to occur;
- the substantial interlaboratory variation of the ferritin value;
- the target value of the serum iron indices during both the depletion and the maintenance phase of phlebotomy treatment.

We therefore recommend world-wide efforts to collaboratively address these issues.
NOTE

The guideline development was initiated by the Netherlands Association of Internal Medicine (NIV), the Netherlands Society of Clinical Chemistry and the Laboratory Medicine (NVKC) and Laboratory Diagnostic Practitioners Association (VAL).
The guidelines were developed within the framework of the EBRO (Evidence Based Guideline Development) Programme of the Order of Medical Specialists in association with the Dutch Society for Gastroenterology (MDL), Dutch College of General Practitioners (NHG), Dutch Blood Transfusion Society (NVB), Dutch Society for Haematology (NVvH), Dutch Society for Pathology (NVVP), Dutch Society for Radiology (NVvR), Dutch Society for Reumatology (NVvR), Dutch Society for Clinical Genetics (VKGN) and the Haemochromatosis Society the Netherlands (HVN). Support was provided by the Committee for Guideline Development of the Netherlands Association of Internal Medicine and the Dutch Institute for Healthcare Quality CBO.
The target audience for this guideline is the Dutch professionals in their management of patients and their relatives with hereditary haemochromatosis, including general practitioners, internists, gastroenterologists, rheumatologists, radiologists, haematologists, clinical pathologists, chemical clinicians and clinical geneticists.

The working party consisted of the following people:

- Dr D.W. Swinkels, clinical chemist and laboratory physician, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, Chairperson
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- Dr R.A. de Vries, gastroenterologist, Rijnstate Hospital, Arnhem, the Netherlands

REFERENCES

Spontaneous fistulisation of a liver abscess into the stomach

Sir,

Treatment of pyogenic liver abscess (PLA) includes antibiotics and drainage. We present a patient with PLA, who developed spontaneous fistulisation into the stomach.

CASE REPORT

A 44-year-old man without a relevant medical history developed abdominal pain. A subsequent upper gastro endoscopy was normal. Abdominal ultrasonography revealed a hypoechoic structure localised in the left hepatic lobe. An abdominal computed tomography (CT) scan (figure 1) demonstrated a 5.5 cm hypodense round structure localised in liver segment II. On admission the body temperature was 36.6°C, and physical examination revealed a painless hepatomegaly. There was a moderate acute phase response. Blood cultures were negative and there was no evidence for presence of echinococcus or amoebiasis on serology. The diagnosis of PLA was made, and a usual antibiotics against pyogenic bacteria was initiated. Six days after admission, an ultrasonographical-guided puncture was planned, but was cancelled, as the PLA was not visible. A CT scan showed that the PLA had drained through a spontaneous fistula into the stomach (figure 2). The outcome was good and the patient was discharged.

CONCLUSION

PLA occurs with an incidence of 22 to 446/100,000 admissions.5,6 Predisposing risk factors are diabetes mellitus, alcoholism, malignancies, immunodeficiency or liver transplantation. The mean age ranges from 50 to 60 years old,6,7 with a male predominance. The main aetiology is cryptogenic, followed by biliary and inflammatory bowel disease.1 The three most observed clinical symptoms are fever, right hypochondrial pain and nausea. A hepatomegaly is found in 25% of cases.2,4 PLA is usually solitary and located in the right liver lobe.5,6 CT scan with contrast media is the gold standard technique to visualise PLA, although ultrasonography is a reliable imaging procedure.5,6 An inflammatory syndrome with leucocytosis and elevation of transaminases are found in two out of three patients.1,2,4 The two most frequent causative organisms are E. coli and K. pneumoniae.1,2 The treatment includes parenteral antibiotics and percutaneous drainages.1,2,4
In our case, drainage was postponed because of initial benefit of the antibiotics, but coincided with spontaneous fistulisation into the stomach, which explains the clinical improvement. This reinforces the concept of early drainage of PLA in order to avoid a spontaneous intra-peritoneal abscess rupture.

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PHOTO QUIZ

Patient with diarrhoea, abdominal pain and weight loss

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CASE REPORT

A previously healthy 59-year-old Caucasian woman presented with intermittent watery diarrhoea, abdominal pain and a 5 kg weight loss in two months. When present, the diarrhoea occurred multiple times during the day and night without blood or mucus. Sometimes she was nauseous and had to vomit. Furthermore, she complained of general malaise.
Laboratory evaluation including electrolytes and liver parameters showed no abnormalities. An ultrasound of the abdomen showed multiple lesions suggestive of metastases in the liver. Later a CT scan was performed (figures 1 and 2).

WHAT IS YOUR DIAGNOSIS?

See page 460 for the answer to this photo quiz.

Figure 1. Abdominal CT scan 1

Figure 2. Abdominal CT scan 2
**DIAGNOSIS**

The CT scan shows a thickened ileum (centre of figure 1) with a tumour mass accompanied by a local desmoplastic mesenteric reaction, i.e. the formation of fibrous tissue, with spiculation of the adjacent mesenteric fat and a calcification (centre of figure 2). These radiological signs are highly suggestive of a carcinoid tumour. Biopsy of a focal liver lesion showed a metastasis of a carcinoid, probably of mid-gut origin, both histologically and immunohistochemically. A SPECT scan using In-111-octreotide performed some days later was positive for both lesions. Urine samples disclosed elevated levels of 5-hydroxyindoleacetic acid. A resection of the affected ileum was performed because of recurrent bowel obstruction. The specimen showed a carcinoid tumour with a diameter of 3 cm. It was penetrating through the wall of the gut. Focal disseminated tumour cells were found in the adjacent fat and lymph nodes.

The patient was treated with long-acting octreotide and thereafter with lutetium-177 (177Lu) octreotate because of persistent diarrhoea.

**REFERENCE**

Blurred vision

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CASE REPORT

A 36-year-old man without relevant medical history was referred to our hospital with classical symptoms of hyperglycaemia. The last few weeks before admission, he had suffered from thirst, polyuria, weight loss, and visual blurring. He was obese with a body weight of 98 kg and height of 1.75 m (BMI 32 kg/m²). Physical examination revealed a few small eruptive xanthomas on his back and left upper leg. Laboratory investigation revealed a grossly elevated blood sugar (32 mmol/l). Fundoscopy was performed (figure 1).

WHAT IS YOUR DIAGNOSIS? WHICH ADDITIONAL LABORATORY TESTS WOULD YOU ORDER?

See page 462 for the answer to this photo quiz.
DIAGNOSIS

The serum of the patient appeared to be extremely lipaemic with serum triglyceride levels of 255 mmol/l (the highest level recorded in this hospital in 30 years), and serum cholesterol 60 mmol/l, indicating massive accumulation of chylomicrons in his blood. Arterial blood gas analysis showed a pH of 7.41 and there were no ketones present in his urine. A variety of laboratory tests was impossible to perform due to the presence of chylomicrons. There were no signs of pancreatitis. Visual acuity was normal 1.0 (OD) and 0.8 (OS).

The patient was treated with intravenous saline and insulin for three days together with metformin 500 mg orally twice daily and withholding food for two days, followed by a low-fat diet afterwards. Shortly thereafter, gemfibrozil 600 mg twice daily was added. His blood glucose levels decreased rapidly within one day to below 10 mmol/l. In five days his serum triglycerides decreased gradually to below 100 mmol/l, and after only three weeks they reached a level just below 3 mmol/l (figure 2). Two months later he was normoglycaemic (blood glucose 5.6 mmol/l, HbA1c 6.2%) with metformin treatment only. His serum triglycerides completely normalised (1.1 mmol/l). Fundoscopy showed no abnormalities (figure 3).

The diagnosis is lipaemia retinalis associated with severe hypertriglyceridaemia caused by de novo diabetes mellitus type 2.
Aims and scope
The Netherlands Journal of Medicine publishes papers in all relevant fields of internal medicine. In addition to reports of original clinical and experimental studies, reviews on topics of interest or importance, case reports, book reviews and letters to the editor are welcomed.

Manuscripts
Manuscripts submitted to the Journal should report original research not previously published or being considered for publication elsewhere. Submission of a manuscript to this Journal gives the publisher the right to publish the paper if it is accepted. Manuscripts may be edited to improve clarity and expression.

Language
The language of the Journal is English. English idiom and spelling is used in accordance with the Oxford dictionary. Thus: Centre and not Center, Tumour and not Tumor, Haematology and not Hematology.

Submission
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Divide the manuscript into the following sections: Title page, Abstract, Keywords, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables and Figures with Legends.

The Title page should include authors’ names, degrees, academic addresses, correspondence address, including telephone number, fax number, e-mail address and grant support. Also the contribution of each author should be specified.

The title should be informative and not exceed 90 characters, including spaces. Avoid use of extraneous words such as ‘study’, ‘investigation’ as well as priority claims (new, novel, first). Give a running title of less than 50 characters. If data from the manuscript have been presented at a meeting, list the name, date and location of the meeting and reference and previously published abstracts in the bibliography. Give a word count (including references, excluding tables and legends) at the bottom of this page.

The Abstract, not exceeding 250 words, should be written in a structured manner and with particular care. In original articles, the Abstract should consist of the following paragraphs: Background, Methods, Results and Conclusion. They should briefly describe the problem being addressed in the study, how the study was performed and which measurements were carried out, the most relevant results, and what the authors conclude from the results.

Keywords: Include three to five keywords.

The Introduction should be brief and set out the purposes for which the study has been performed.

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Acknowledgement: All funding sources should be credited here. Also a statement of conflicts of interest should be mentioned.
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Journal abbreviations should conform to the style used in the Cumulated Index Medicus. Examples:


Please note that all authors should be listed when six or less; when seven or more, list only the first three and add et al. Do not include references to personal communications, unpublished data or manuscripts either ‘in preparation’ or ‘submitted for publication’. If essential, such material may be incorporated into the appropriate place in the text. Recheck references in the text against the reference list after your manuscript has been revised.

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Tables should be typed with double spacing each on a separate page, numbered consecutively with Arabic numerals, and should contain only horizontal lines. Provide a short descriptive heading above each table with footnotes and/or explanation underneath.

Figures must be suitable for high-quality reproduction (>300 DPI). Submit line drawings made in Word or other computer programmes but not in a PowerPoint file. Colour figures are occasionally possible and will be charged to the authors.

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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.

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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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Photo quiz

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