PHOTO QUIZ: Blurred vision, see page 461

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EDITORIAL INFORMATION

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EDITORIAL

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 HH[2] 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 athropathy 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, nonhereditary 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.

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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 sulphalazine[4] 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.[8,9] 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.[11-13] The most widely used probiotics in humans are *Bifidobacteria* and *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.[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.

MATERIALS AND METHODS

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.

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.[15,16] In one trial the two groups of patients receiving anti-inflammatory drugs were considered as one control group.[15] The second trial included two probiotic groups.[16] Each one of them was compared with the control group separately.

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.

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.

RESULTS

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,[19,21,29,30,36] four referred to pouchitis,[19,28,33,34] one referred to inflammatory bowel disease,[23] one to colonic surgery,[32] three to Crohn's disease[25,26,38] and one[27] 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 synbiotic compared with placebo in patients with active UC.[22] One study used balsalazide and VSL#3 compared with mesalazine and balsalazide in patients with mild to moderate UC.[15] One study used *Lactobacillus* GG compared with mesalazine and with *Lactobacillus* GG plus mesalazine.[16] Three studies used *E. coli* compared with mesalazine in active and in inactive UC[18,20,31] and

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

Authors	Year	Disease	Randomised controlled trial	Probiotic
Tursi et al.[15]	2004	Ulcerative colitis	Yes	Yes
Zocco et al.[16]	2006	Ulcerative colitis	Yes	Yes
Ishikawa et al.[17]	2002	Ulcerative colitis	Yes	Yes
Kruis et al.[18]	2004	Ulcerative colitis	Yes	Yes
Braegger et al.[19]	2003	Pouchitis	No	Yes
Rembacken et al.[20]	1999	Ulcerative colitis	Yes	Yes
Bibiloni et al.[21]	2005	Ulcerative colitis	No	Yes
Furrie et al.[22]	2005	Ulcerative colitis	Yes	Yes
Annese et al.[23]	2004	Inflammatory bowel disease	Yes	No
Kato et al.[24]	2004	Ulcerative colitis	Yes	Yes
Schultz et al.[25]	2004	Crohn's disease	Yes	Yes
Teml et al.[26]	2003	Crohn's disease	Yes	No
Cui et al.[27]	2003	Ulcerative colitis	Yes	Yes
Gionchetti et al.[28]	2000	Pouchitis	Yes	Yes
Folwaczny[29]	2000	Ulcerative colitis	No	Yes
Faubion et al.[30]	2000	Ulcerative colitis	No	Yes
Kruis et al.[31]	1997	Ulcerative colitis	Yes	Yes
Everett et al.[32]	1969	Colonic surgery	Maybe	No
Kuhbacher et al.[33]	2006	Pouchitis	Maybe	Yes
Gionchetti et al.[34]	2003	Pouchitis	Yes	Yes
Cui et al.[35]	2004	Ulcerative colitis	Yes	Yes
Bai et al.[36]	2006	Ulcerative colitis	No	Yes
Shibata et al.[37]	2007	Ulcerative colitis	Yes	Yes
Van Gossum et al.[38]	2007	Crohn's disease	Yes	Yes

three studies used *Bifidobacteria* compared with placebo in mild to moderate and in active UC.[17,24,35] 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.[17,35] Two studies showed a trend for increased efficacy and five trials did not show any significant difference between probiotic and control groups.[15,22] 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 significant heterogeneity was found (Q=28.61). The normal heterogeneity for 9 degrees of freedom (df) according to the χ^2 distribution is 14,684.

Adverse effects
Seven of the nine (77.8%) trials presented data on adverse reactions.[15,17,18,20,22,24,31] The pooled relative risk of adverse effects for the seven studies was: 1.17 (0.81-1.70), p=0.40.

A nonsignificant heterogeneity was found (Q=5.47). The normal heterogeneity for 6 df according to the χ^2 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.[22] The other two studies had a trend for increased efficacy.[15,24] 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 χ^2 distribution is 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.[17,35] The other four trials did not find any significant difference between the probiotic and control group.[16,18,20,31] 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 significant heterogeneity was found (Q=24.26) as the normal heterogeneity for 6 df according to the χ^2 distribution was 10,645.

Table 2. *Characteristics of nine randomised controlled trials assessing the effect of probiotics in ulcerative colitis remission*

Authors, year	Probiotic	Control group	Dose (n of probiotic/day)	Treatment duration	N (probiotic/control group)	Disease severity	Induction or maintenance of remission N (probiotic/control group)	Outcome measures
Tursi et al.[15] 2004	Balsalazide/VSL#3	Mesalazine/balsalazide	900 x 10^8	8 weeks	30/30/30	Mild-to-moderate	Induction of remission 24/21/16	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
Zocco et al.[16] 2006	*Lactobacillus* GG	Mesalazine	18 x 10^9	12 months	65/60/62	Inactive UC	Maintenance of remission 55/48/52	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
Ishikawa et al.[17] 2002	*Bifidobacterium breve Bifidobacterium bifidum Lactobacillus acidophilus* YIT 0168	BFM without these *Bifidobacteria*	10 x 10^8	12 months	11/10	Mild Moderate	Maintenance of remission 8/1	Exacerbation of clinical symptoms
Kruis et al.[18] 2004	*E. coli* Nissle 1917	Mesalazine	2.5-25 x 10^9	12 months	162/165	Inactive	Maintenance of remission 89/104	Comparison of number of patients with relapse of UC between the two groups
Rembacken et al.[20] 1999	*E. coli* Nissle 1917 serotype O6: K5: H1	Mesalazine	5 x 10^10	12 months	57/59	Active	Maintenance of remission 39/44	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
Furrie et al.[22] 2005	Synbiotic (*Bifidobacterium longum* + inulin-oligofructose)	Potato starch and sachet of 6 g powdered maltodextrose	4 x 10^11	4 weeks	9/9	Active	Induction of remission 5/3	1. Clinical improvement in symbiotic *vs* placebo group 2. Effects of symbiotic in mucosa
Kato et al.[24] 2004	*Bifidobacterium breve* strain Yakult *Bifidobacterium bifidum* strain Yakult *Lactobacillus acidophilus*	BFM without *B. bifidum* and *L. acidophilus*	10^9	12 weeks	10/10	Mild-to-moderate active	Induction of remission 4/3	Clinical improvement (indicated by a decrease in CAI score of ≥3 points)
Cui et al.[27] 2004	*Bifidobacteria*	Starch	1.26 g/d	8 weeks	15/15	Active	Maintenance of remission 12/1	Effects of probiotics on intestinal mucosae and role of probiotics in preventing relapse of UC
Kruis et al.[31] 1997	*E. coli* Nissle 1917 serotype O6: K5: H1	Mesalazine	50 x 10^9	12 weeks	50/53	Inactive UC	Maintenance of remission 42/51	Prove equivalence of the CAI score under the *E. coli* and mesalazine

N = number of patients.

Table 3. *Methodological quality of the nine studies*

Authors, year	Inclusion/ exclusion criteria	Description of the patients	Co-treatment/con- comitant medication (probiotic group)	Outcome measurement
Tursi *et al.*[15] 2004	Both	Patients with newly diagnosed or recently relapsed (within 4 weeks) mild-to-moderate UC confirmed by endoscopic evaluation	Probiotic + balsalazide	CAI by Lennard/EI score/ HI score
Zocco *et al.*[16] 2006	Both	Patients with UC in clinical, laboratory and endoscopic remission of ≤12 months before admission	1 group: probiotic 2 group: probiotic + mesalazine	CAI according to Rachmilewitz/EI by Baron/ HI by Truelove-Richard
Ishikawa *et al.*[17] 2002	Inclusion criteria	Patients who had been diagnosed with UC ≥1 year previously based on clinical grounds and colonoscopic findings	Probiotic only	Not mentioned
Kruis *et al.*[18] 2004	Both	Patients aged 18-70 with UC in remission (CAI ≤4, EI ≤4 and no signs of acute inflammation on histological examination)	Probiotic only	Scores according to Rachmilewitz
Rembacken *et al.*[20] 1999	Inclusion criteria	Patients aged 18-80 years with clinically active UC (≥4 liquid stools a day for the last 7 days with or without blood) with at least erythema on sigmoidoscopy and histological confirmation of active UC	Probiotic only	Scores according to Rachmilewitz
Furrie *et al.*[22] 2005	Inclusion criteria	Patients with active UC aged 24-67 who had not received antibiotics in the last 3 months and were not taking commercially available probiotic preparations	Probiotic + prebiotic (synbiotic)	CAI by Walmsley/SI by Baron
Kato *et al.*[24] 2004	Both	Patients with active UC; diagnosis confirmed by characteristic clinical, endoscopic and histological features	Probiotic only	CAI by Lichtiger/EI by Harig, Scheppach/HI by Matts
Cui *et al.*[27] 2004	Not mentioned	Active UC patients	Probiotic only	Not mentioned
Kruis *et al.*[31] 1997	Both	Patients >17 years, presence of chronic UC, previously diagnosed by endoscopic and histological criteria and now in remission	Probiotic only	Scores according to Rachmilewitz

CAI = clinical activity index; SI = sigmoidoscopy index; EI = endoscopy index; HI = histology index.

Table 4. *Total random effects (clinical success and adverse effects) from nine randomised controlled trials*

Outcome	Patients (n)	Probiotic		Control		Odds ratio	95% CI		p	Q	p (Q)
		N in remission	Total	N in remission	Total		Low	High			
Clinical success	972	330	471	340	501	1.51	0.79	2.87	0.21	28.61	14,684
Adverse effects	710	86	337	83	373	1.17	0.81	1.70	0.40	5.47	10,645

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.[24] The other four studies did not find any significant difference between probiotics and anti-inflammatory agents.[16,18,20,31] The pooled relative risk was 0.95 (95% CI 0.58-1.55, 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 χ^2 distribution was 9236.

Among four randomised, controlled studies with probiotics with placebo, two trials reported significantly higher remission in UC for patients receiving probiotics.[17,35] The other two trials showed a trend for increased efficacy of probiotic compared with placebo.[22,24] The pooled relative risk was 7.32 (95% CI 1.37-39.13, 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 3.79 (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.[8,39] 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.[24] 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.[17] 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.[40] 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* recolonised, and relapses occurred in some patients.[41] 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 conrol group)[17,24,35] showed better results than the trials with patients who did not use antibiotics.[15,16,22] 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.[41] Compared with many pharmaceutical agents, serious adverse effects from probiotics rarely occur because they are well tolerated and safe.[42] 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.[43] 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,[39] Souza *et al.* on antibiotic associated diarrhoea,[44] van Niel *et al.* on acute infectious diarrhoea in children.[45] Sazawal *et al.* on acute diarrhoea[46] and McFarland on antibiotic-associated diarrhoea.[41] 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.[47,48] A meta-analysis by Szajewska and Mrukowicz found moderately effective results for *Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhoea.[49]

A micro-organism classified as a probiotic has to have the following properties: exhibit 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.[46] 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.[39] The way in which probiotics affect the gut is of much interest. To overcome the problems of gastrointestinal infection, a probiotic must be nonpathogenic 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.[45]

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.

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REVIEW

Changing aspects of *HFE*-related hereditary haemochromatosis and endeavours to early diagnosis

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ABSTRACT

HFE-related hereditary haemochromatosis (HH) is an iron overload disease attributed to the highly prevalent homozygosity for the C282Y mutation in the *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 *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 *HFE*-related iron overload. Over time it appeared that the clinical penetrance of the *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 *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.

KEYWORDS

Diagnosis, family, hereditary haemochromatosis, *HFE*, screening

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 *HFE* gene, large-scale screening for *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 *HFE* gene mutation. This review describes new insights into pathophysiology, diagnosis and penetrance of *HFE*-related HH, and its implications for secondary prevention and early treatment of the clinical disease.

HISTORY

One of the first to describe a clinical syndrome characterised by cirrhosis of the liver, diabetes mellitus

and bronze skin pigmentation was Trousseau.[1] The name haemochromatosis was first used by von Recklinghausen (1889), describing post-mortem findings in patients who had died from 'bronzed diabetes'.[2] In 1935, Sheldon suggested a familial form of haemochromatosis,[3] 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.[4] 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.[5]

Until now, more than 30 allelic variants of the *HFE* gene have been reported.[6] 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.[7-9] 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).[4] 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.[10] In North America an overall frequency of C282Y heterozygosity, regardless of the ethnical 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.[10] For the Netherlands the percentages of C282Y homozygosity and heterozygosity are calculated at 0.2 and 12.0%, respectively.[11]

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.[12] 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.[13] 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.[14] 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.[15] 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.[16-19]

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'.[20] 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.[20-27] 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.[28-30] 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

Figure 1. *Schematic view of the five stages of HFE-related hereditary haemochromatosis (HH) together with the various strategies for screening on HH*[34]

Five stages	Composition of the population
C282Y homozygosity with increased transferrin saturation, increased serum ferritin, and clinical symptoms manifesting organ damage predisposing to early mortality	IV
C282Y homozygosity with increased transferrin saturation, increased serum ferritin, and clinical symptoms affecting the quality of life	III
C282Y homozygosity with both increased transferrin satura-tion, increased serum ferritin, but no clinical symptoms	II
C282Y homozygosity with increased transferrin saturation, but normal serum ferritin values and no clinical symptoms	I
C282Y homozygosity without biochemical or clinical symptoms (normal plasma transferrin saturation and serum ferritin)	O

Green = population screening strategy; yellow = family screening strategy; red = individuals who develop clinically important HH, targeted screening; horizontal red bar = individuals with C282Y homozygosity.

for increased body iron levels. However, abnormal values can be found in the presence of other pathology, including liver diseases and alcohol abuse.[31-35] Homozygosity for the C282Y mutation or the combined C282Y/H63D genotype in the *HFE* gene analysis confirms the HH diagnosis.[34,36] 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.[37,38] Hepatic magnetic resonance imaging (MRI) provides a noninvasive approach to semi-quantify the amount of liver iron.[39-41] The severity of iron overload can also be calculated from the number of phlebotomies required to deplete iron stores.[20,22]

TREATMENT OF HEREDITARY HAEMOCHROMATOSIS

The treatment of HH consists of venesection, as described by Davis.[42] 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.[31-35] 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.[20,32,43] 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.[43-47] Consumption of black tea with meals has been reported to decrease iron absorption by formation of nonabsorbable iron complexes.[48]

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.*[49] 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.[49] 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*).[50,51] Many reports have been written on the feasibility of early screening on HH in the general population.[52-61] 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.[50,51,53,62]

Table 1. *Restatement of the Wilson and Jungner principles for mass screening programmes (World Health Organization, 1968)[50]*

1. The condition being sought should be an important health problem, for the individual and the community
2. There should be an acceptable form of treatment for patients with recognisable disease
3. The natural history of the condition, including its development from latent to declared disease, should be adequately understood
4. There should be a recognisable latent or early symptomatic stage
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
6. The facilities required for diagnosis and treatment of patients revealed by the screening programme should be available
7. There should be an agreed policy on whom to treat as patients
8. Treatment at the presymptomatic, borderline stage of a disease should favourably influence its course and prognosis
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
10. Case finding should be a continuing process and not a 'once and for all' project

One important question that remains unanswered: Is HH indeed an important health problem, for the community, and for the individual?[50,51,63] At first it was assumed that all C282Y homozygous individuals would eventually develop iron overload resulting in tissue damage and disease.[31] 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.[21,49,64-67] 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.[29,30,68-71]

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.[35,72,73] 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.[74] Implementation of a guideline for physicians on the targeted detection of HH in an early, symptomatic, stage could be beneficial.[72] 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.[75]

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.[28] 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.[76-79]

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 Beutler and 5% found by Powell.[29,49,63]

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 instrumental answers on how to prevent disease in as yet unidentified individuals at risk for *HFE*-related HH.

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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ORIGINAL ARTICLE

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, morality

INTRODUCTION

HFE-related hereditary iron overload is characterised by iron deposition in parenchymal organs.[1,2] Early detection and phlebotomy prevent tissue damage and result in long-term survival similar to that in the general population.[2-6] 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

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.

persons.[7] 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.[8-11] Therefore, family screening has been suggested, since this has proven efficacy in the detection of latent homozygotes for frequent recessive mutations.[12] 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?[13] However, to date, to our knowledge there is no such a study that has extensively compared the morbidity and mortality in *HFE*-related HH families with the morbidity and family-related mortality of a general, apparently healthy, population, whereas these outcomes are required to legitimate further research on the implementation of family screening.

Therefore, the objective of the present study was to compare self-reported morbidity among first-degree family members (FDFM) of C282Y-homozygous probands previously diagnosed with clinically manifest HH, with data obtained from age- and gender-matched controls from a normal population. Furthermore the mortality rates among FDFM in these HH families, as reported by the HEFAS probands, were compared with the mortality among the FDFM of age- and gender-matched participants from the normal population. Notably, the study is observational and descriptive and not designed to explain the similarities and differences in outcomes of 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.[14]

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 relatives (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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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).[1,15]

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, vitality[16] and fatigue[17] were used for further evaluation.

Haemochromatosis-related medication use was calculated by counting the use of (1) analgesics, (2) antirheumatic 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 (1) 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 of 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

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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 1. *Size and structure of the families of the HEmochromatosis FAmily Study (HEFAS) probands*

		Siblings						
		0	1	2	3	4	≥5	*Total*
Children:	0	45	17	15	9	10	9	105
	1	13	9	6	7	3	6	44
	2	13	7	7	8	4	7	46
	3	6	3	0	5	3	2	19
	4	1	1	1	2	1	0	6
	≥5	0	1	1	0	2	0	4
Total		78	38	30	31	23	24	224
Both parents		5	3	3	1	3	3	18
Father or mother		3	4	9	6	2	6	30
No parents		70	31	18	24	18	15	176

Table 2. *Characteristics of the first-degree family members of the HEFAS probands and of the age- and gender-matched NBS participants*

	HEFAS		NBS		HEFAS - NBS	
	Total	Median (range)/n (%)	Total	Median (range)/n (%)	Total[*]	Difference[#](%) (95% CI)
Demographics:						
• Age at participation (years)	735	48 (18-97)	735	48 (18-97)	735	n.a.
• Men	735	318 (43.3)	735	318 (43.3)	735	n.a.
• Education (≥secondary)	689	198 (28.7)	732	285 (38.9)	686	-9.9 (-14.5; -5.3)
• Household (single with or without children)	723	136 (18.8)	734	230 (31.3)	722	-12.3 (-16.4; -8.3)
• Paid job (≥32 hrs/week)	342	185 (54.1)	458	208 (45.4)	290	2.8 (- 4.8; 10.3)
Lifestyle:						
• Alcohol (>2 units/day)	628	163 (26.0)	702	234 (33.3)	602	-8.3 (-13.2; -3.4)
• Smoking (ever)	727	463 (63.7)	733	460 (62.8)	725	0.8 (- 0.4; 5.7)
Blood loss:						
• Blood donation (never)	705	560 (79.4)	727	544 (74.8)	698	4.3 (0.0; 8.7)
• ♀Menarche (≤12 years)	403	128 (31.8)	404	127 (31.4)	391	-0.7 (-7.4; 5.9)
• ♀Pregnancies (>3)	417	79 (18.9)	417	51 (12.2)	417	6.7 (2.0; 11.5)

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.

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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*

	HEFAS		NBS		HEFAS - NBS	
	Total	Median (range)/n (%)	Total	Median (range)/n (%)	Total[*]	Difference[#](%) (95% CI)
Body mass index (kg/m²)	717	24.9 (15.2-60.6)	718	24.4 (16.9-62.4)	701	1.7 (0.1; 2.4)
General health:						
• Exercise (≤1 hr/week)	415	109 (26.3)	412	151 (36.7)	250	-4.4 (-12.0; -3.2)
• Health (>2)[‡]	722	204 (28.3)	733	162 (22.1)	720	6.5 (2.2; 10.9)
• General mental health last 4 weeks (≤23)[§]	684	339 (49.6)	697	461 (51.8)	650	-1.7 (-7.1; 3.7)
• Physical functioning at this moment(≤23)[##]	656	108 (16.5)	686	72 (11.5)	617	6.0 (2.5; 9.5)
• Vitality last 4 weeks (≤17)[¥¥]	680	376 (55.3)	701	325 (46.4)	649	9.1 (3.7; 14.4)
Medication used (yes):						
• Analgesics	627	321 (51.2)	691	285 (41.2)	593	9.8 (4.1; 15.4)
• Antihypertensive drugs	654	146 (22.3)	690	94 (13.6)	617	8.8 (4.9; 12.6)
• Antirheumatic drugs	601	63 (10.5)	673	35 (5.2)	556	5.9 (2.9; 9.0)
• Cardiovascular drugs	614	70 (11.4)	681	50 (7.3)	574	4.4 (1.2; 7.5)
• Diuretics	606	73 (12.0)	683	61 (8.9)	572	3.2 (0.1; 6.2)
• Folic acid	583	67 (11.5)	655	61 (9.3)	531	2.4 (-1.0; 5.8)
• Lipid-lowering drugs	614	57 (9.3)	682	48 (7.0)	576	3.0 (0.1; 5.8)
• Iron supplements	718	87 (12.1)	674	141 (20.9)	659	-9.0 (-12.7; -5.2)
• Tranquillizers	618	148 (24.0)	696	150 (21.6)	590	3.0 (-1.5; 7.6)
• (Multi)vitamin preparations	613	221 (36.0)	675	199 (29.5)	570	6.0 (0.5; 11.5)
• Vitamin B complex	593	139 (23.4)	668	124 (18.6)	542	5.5 (1.0; 10.2)
• Vitamin C complex	601	197 (32.8)	670	174 (26.0)	556	7.9 (2.7; 13.1)
Haemochromatosis-related medication (analgesics, antirheumatic drugs and cardiovascular medication)	677	421 (62.2)	708	348 (49.2)	652	13.3 (8.2; 18.4)
Morbidity[¥]:						
• Anaemia	620	99 (16.0)	674	90 (13.4)	575	3.0 (-1.1; 7.0)
• Cancer	621	35 (5.6)	683	48 (7.0)	584	-1.4 (-4.1; 1.4)
• Cardiovascular disease	620	65 (10.5)	685	28 (4.1)	582	5.5 (2.7; 8.3)
• Cerebrovascular accident	604	17 (2.8)	675	9 (1.3)	561	1.4 (-0.1; 3.0)
• Diabetes mellitus	620	25 (4.0)	677	31 (4.6)	574	0.4 (-1.8; 2.5)
• Fatigue (≥18)[**]	688	90 (13.1)	683	54 (7.9)	643	5.9 (2.7; 9.1)
• Hypercholesterolaemia	623	97 (15.6)	684	80 (11.7)	582	4.5 (0.8; 8.1)
• Hypertension	648	184 (28.4)	689	138 (20.0)	609	7.9 (3.5; 12.3)
• Infertility	604	22 (3.6)	669	28 (4.2)	557	-0.5 (-3.0; 1.9)
• Liver disease	611	31 (5.1)	669	17 (2.5)	563	3.2 (1.0; 5.4)
• Osteoporosis	612	47 (7.7)	677	25 (3.7)	570	4.2 (1.8; 6.6)
• Rheumatism	638	199 (32.2)	678	41 (6.0)	594	24.6 (20.6; 28.6)
• Surgery	722	499 (69.1)	728	482 (66.2)	715	2.6 (-2.2; 7.2)
• Thyroid disease	610	28 (4.6)	671	30 (4.5)	565	0.0 (-2.4; 2.4)
Haemochromatosis-related diseases (diabetes mellitus, liver disease, rheumatism, fatigue and cardiovascular disease)	652	298 (45.7)	675	131 (19.4)	599	25.7 (20.9; 30.5)
Iron parameters[†]:						
• Serum transferrin saturation >50%	599	176 (29.4)	494	21 (4.2)	403	25.3 (20.5; 30.1)
• Serum ferritin above normal (μmol/l)[‡‡]	487	198 (40.7)	409	106 (21.2)	333	16.5 (9.7; 23.3)
• Serum transferrin saturation (%)[§§]	207	38.4 (3.2-107.3)	135	29.5 (4.8-97.7)	135	37.1 (23.4; 52.5)
• Serum ferritin (μmol/l)[§§]	207	119.0 (4.0-2308)	137	93.9 (6.6-4737)	137	32.4 (7.4; 63.1)

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] [##]10: bad physical functioning to 30: good physical functioning, using the SF-36 health survey score;[16] [¥¥]4: low vitality to 24: high vitality, using the SF-36 health survey score;[16] [¥]self-reported diagnosis of morbidity made by a physician; [**]4: fatigue absent to 24: fatigue present, using the shortened fatigue questionnaire score;[17] [†]at time of being tested for hereditary haemochromatosis; [‡‡]serum ferritin above the local upper reference value; [§§]only participants tested in the Radboud University Nijmegen Medical Centre.

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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%: 1.7%, 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

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)*
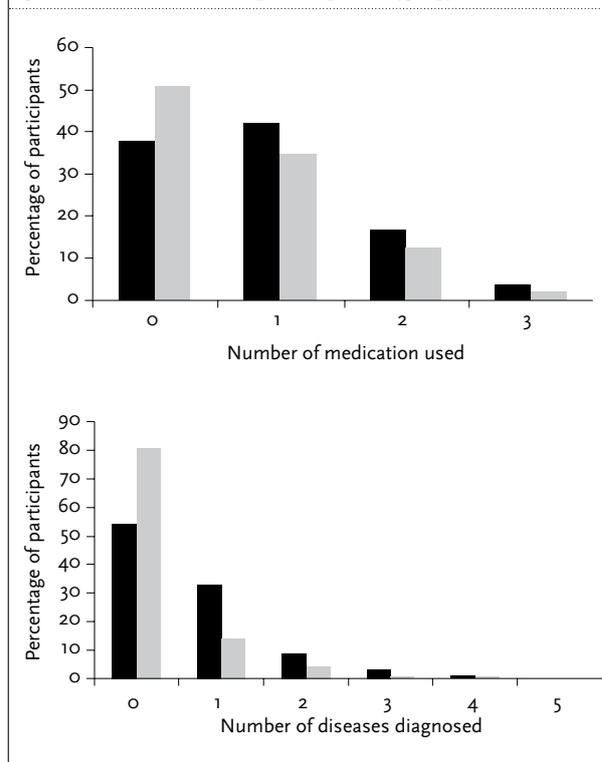
participants, with a difference between HEFAS and NBS for TS of 25.3% and for SF of 16.5% (*table 3*). Similarly, the relative differences in the absolute values of TS and SF between the FDFM of the HEFAS and the matched NBS participants were 37.1 and 32.4%, respectively, using only the samples measured in the RUNMC.

Figure 2 shows both the amount of haemochromatosis-related medication use and number of diseases of the FDFM of the HEFAS population and the age- and gender-matched NBS participants. A significantly higher percentage of FDFM used haemochromatosis-related

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

	HEFAS			NBS			
	Families	n	Deceased n (%)	Families	n	Deceased n (%)	P value[*]
Parents	224	427	299 (70.0)	224	421	310 (73.6)	0.25
Siblings	224	709	93 (13.1)	224	752	99 (13.2)	1.00
Children	224	414	8 (1.9)	224	372	5 (1.3)	0.59

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.

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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 224 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 arthropathy 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.[1,2-5] 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.[18-21] 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.[22-24] 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.[23] 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

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by altering immunological processes, all leading to an advantage in survival.[3,26-29] 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.[30] 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.[31] 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 HEFAS 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

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ORIGINAL ARTICLE
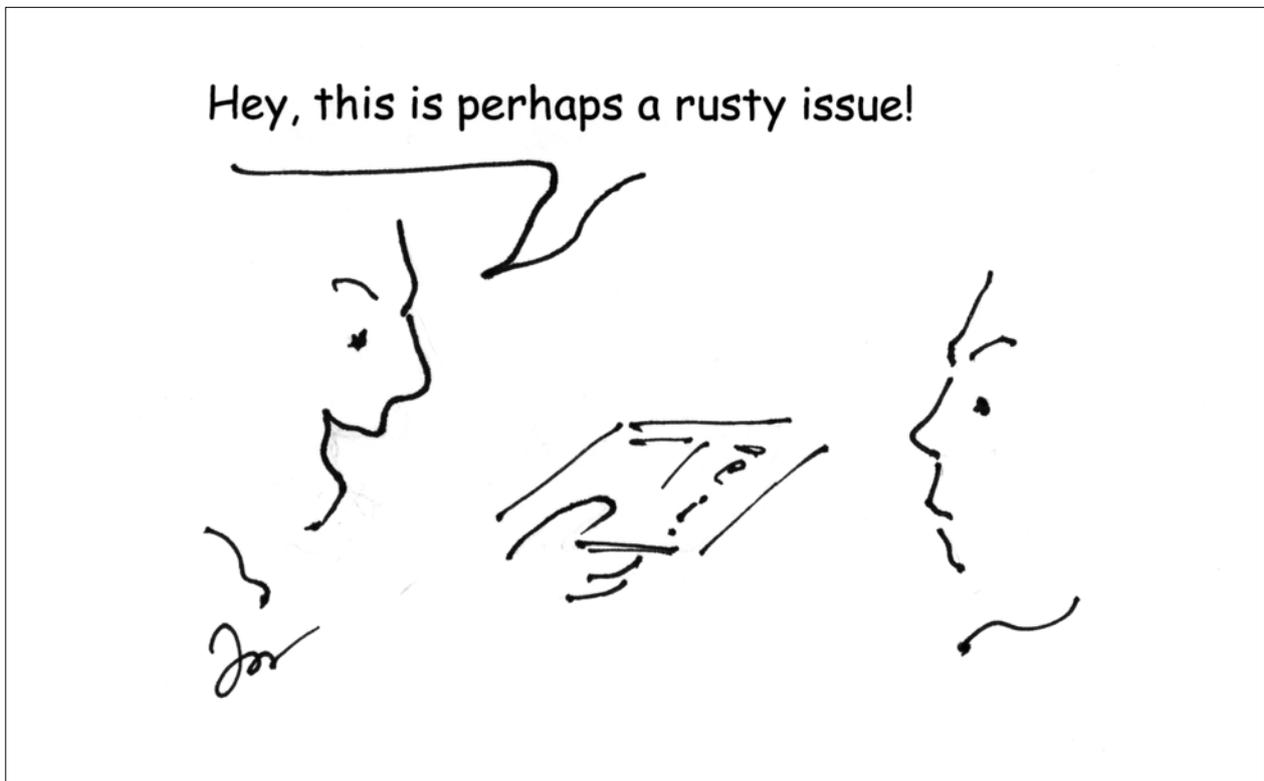
Incidence of first acute myocardial infarction in the Netherlands

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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,[1] 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,[2] cohort studies,[3-5] and from linkage of regional registries.[6-10] Only a few countries provide nationwide data on the incidence of AMI.[6-10] In the Netherlands the incidence of AMI is derived from local primary care registries[11] and mortality and hospital discharge rates for AMI were traditionally frequently examined for the Netherlands using national registries.[1,12,13] 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,[14] 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).[15] 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)[14] and constant over time. The numeric part of postal code, however, can change when patients move (estimated rate of 6% per year).[5] 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[16] (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.[14]

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[16] 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*).

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Figure 1. *Flowchart of the selection process of patients with a first hospitalised acute myocardial infarction (AMI) in 2000 in the Netherlands*

All hospital admissions in HDR with principal diagnosis AMI in 2000
(n=24,954)

Linkage with PR

Admissions of patients with a unique code in the PR in 2000
(n=22,470)

Admissions from patients with a non-unique code in the PR in 2000 (n=2484)

Selection first admission per patient during 2000

First admission per patient during 2000
(n=20,414)

Readmission(s) during 2000
(n=2056)

linkage with HDR*PR 1995-2000

Patients without previous admissions for AMI during 1995-2000 (n=19,058)

Patients with previous admissions for AMI during 1995-2000 (n=1356)

Patients with a first hospitalised AMI in 2000
(n=19,058)

HDR = Dutch National Hospital Discharge Register, PR = Dutch Population Register.

Between 1 January and 31 December 2000, a total of 16,941 deaths with as primary cause of death AMI (ICD-10[16] code I21) or other ICD-10[7] 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.[17] The proportion of out-of-hospital deaths of the total number of first AMI

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Figure 2. *Flowchart of the selection process of out-of-hospital deaths from a first acute myocardial infarction (AMI) in 2000 in the Netherlands*


```
All deaths in causes of death register with primary cause of death indicating an AMI in 2000
(n=16,941)
                                    │
        Linkage with cohort patients with
        a first hospitalised AMI in 2000 ───────────────────────┐
                                    │                            │
        Out-of-hospital deaths in 2000          Hospitalised deaths in 2000
                (n=14,578)                              (n=2363)
                                    │
        Linkage with PR ─────────────────────────────────────────┐
                                    │                            │
        Deaths with a unique code in the PR in 2000   Deaths with a non-unique code in the PR in 2000
                (n=13,368)                              (n=1210)
                                    │
        Linkage with HDR*PR
        1995-2000 ───────────────────────────────────────────────┐
                                    │                            │
        Patients without previous admissions     Patients with previous admissions
        for AMI during 1995-2000 (n=12,719)       for AMI during 1995-2000 (n=649)
                                    │
        Out-of-hospital deaths from a first AMI in 2000
                (n=12,719)
```

HDR = Dutch National Hospital Discharge Register, PR = Dutch Population Register.

patients was computed by age and gender. Within the age groups, the difference in proportion of out-of-hospital deaths between men and women was tested with the χ^2 test for homogeneity of proportions. A p value less than 0.05 was considered statistically significant.

RESULTS

In 2000, we identified 31,777 patients with a first AMI of whom 19,058 were hospitalised (60%) and 12,719 died out of hospital (40%). Two-thirds of the hospitalised patients (*table 1*) and 55% of the out-of-hospital deaths (*table 2*) were men. The patients dying out of hospital were on average 9 years older than patients dying in hospital. The age of women at admission was on average 7 to 8 years higher than that of men. The mean length of hospital stay was ten days. During admission, 12% of the patients died (men 10%, women 17%). In both the hospitalised patients and the out-of-hospital deaths, most patients were native Dutch (88-90%). AMI was the primary cause of death in 72% of the out-of-hospital deaths, cardiac arrest in 21% and sudden death with unknown cause in 6%.

Table 1. *Characteristics of patients with a first hospitalised acute myocardial infarction in the Netherlands in 2000*

	Men	Women	Total
Number of patients	12,783	6275	19,058
Age at admission (years):			
• Mean (SD)	64.2 (12.7)	71.6 (12.8)	66.7 (13.2)
• Median	65.0	73.8	68.0
Type of hospital (%):			
• University	7.5	6.2	7.0
• Peripheral	92.5	93.8	93.0
Length of stay (days):			
• Mean (SD)	8.9 (8.4)	10.0 (10.7)	9.2 (9.3)
• Median	7.0	8.0	8.0
• 25th-75th percentile	5.0-10.0	5.0-12.0	5.0-11.0
Origin (%):			
• Native	88.3	88.4	88.4
• Non-native	11.7	11.6	11.6

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

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Table 2. *Characteristics of out-of-hospital deaths from a first acute myocardial infarction in the Netherlands in 2000*

	Men	Women	Total
Number of patients	6972	5747	12,719
Age at death (years):			
• Mean (SD)	72.0 (13.2)	80.0 (11.5)	75.6 (13.1)
• Median	74.0	82.1	77.9
Origin (%):			
• Native	90.4	89.7	90.1
• Non-native	9.6	10.3	9.9
Primary cause of death (ICD-10[8] code) (%):			
• Acute myocardial infarction (I21)	73.6	70.5	72.1
• Subsequent myocardial infarction (I22)	0.1	0.2	0.1
• Current complications following acute myocardial infarction (I23)	0.0	0.0	0.0
• Other forms of acute ischaemic heart disease (I24.8)	0.2	0.4	0.3
• Unspecified acute ischaemic heart disease (I24.9)	1.0	1.2	1.2
• Cardiac arrest (I46)	20.2	21.3	20.7
• Sudden death with unknown cause (R96)	4.9	6.4	5.6

women from 1 (95% CI 0.5 to 1.2) to 2226 (95% CI 2100 to 2351) in the corresponding age groups. In all age groups, the incidence of both hospitalised and non-hospitalised first AMI was higher in men than in women (*table 4*). This was most pronounced in the age group 50-59 years, in which the incidence was four times higher in men compared with women. After the age of 50-59 years, the male-to-female ratio decreased, indicating a relatively high increase in incidence of women older than 50-59 years.

The proportion of out-of-hospital deaths from a first AMI of the total number of first AMI patients increased with age in men after the age of 40-49 years (from 19 to 85%) and in women after the age of 50-59 years (from 21 to 82%) (*figure 3*). There was no statistically significant difference in proportion of out-of-hospital deaths between men and women within the age groups. In the age groups up to 70-79 years, the majority of first AMI patients were hospitalised, in the age groups 80-89 years and ≥90 years most patients died before being admitted to a hospital.

DISCUSSION

Our study provides estimates of the incidence of patients with a first AMI in the Netherlands. These estimates are based on linkage of Dutch national registries and represent for the first time virtually the whole Dutch population. An increasing incidence with age and a higher incidence in

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

	Age (years)	Hospitalised patients	Out-of-hospital deaths	Total number of first AMI cases	Total number of persons[*]	Incidence	95% CI
Men	<30	37	21	58	2,576,315	2	2-3
	30-39	356	98	454	1,098,227	41	38-45
	40-49	1450	346	1796	1,011,713	178	169-186
	50-59	2960	845	3805	892,870	426	413-440
	60-69	3344	1375	4719	606,735	778	756-800
	70-79	3291	2189	5480	399,574	1371	1335-1408
	80-89	1276	1721	2997	138,020	2171	2094-2249
	≥90	69	377	446	14,886	2996	2718-3274
	All ages	*12,783*	*6972*	*19,755*	*6,738,340*	*293*	*289-297*
Women	<30	13	8	21	2,485,992	1	1-1
	30-39	90	41	131	1,067,573	12	10-14
	40-49	374	102	476	989,158	48	44-52
	50-59	692	186	878	867,587	101	95-108
	60-69	1250	550	1800	637,725	282	269-295
	70-79	2108	1536	3644	531,798	685	663-708
	80-89	1533	2326	3859	283,866	1359	1317-1402
	≥90	215	998	1213	54,502	2226	2100-2351
	All ages	*6275*	*5747*	*12,022*	*6,918,201*	*174*	*171-177*

[*]**Number of unique persons in the PR on 1 July 2000.**

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

Age (years)	Hospitalised first AMI patients		Out-of-hospital deaths from a first AMI		Total number of first AMI patients	
	RR Men/women	95% CI	RR Men/women	95% CI	RR Men/women	95% CI
<30	2.75	1.46-5.17	2.53	1.12-5.72	2.67	1.62-4.39
30-39	3.85	3.05-4.85	2.32	1.61-3.35	3.37	2.77-4.09
40-49	3.79	3.38-4.25	3.32	2.66-4.14	3.69	3.33-4.08
50-59	4.16	3.83-4.51	4.41	3.77-5.17	4.21	3.91-4.53
60-69	2.81	2.63-3.00	2.63	2.38-2.90	2.76	2.61-2.91
70-79	2.08	1.97-2.19	1.90	1.78-2.02	2.00	1.92-2.09
80-89	1.71	1.59-1.84	1.52	1.43-1.62	1.60	1.52-1.68
≥90	1.18	0.90-1.54	1.38	1.23-1.56	1.35	1.21-1.50

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)*
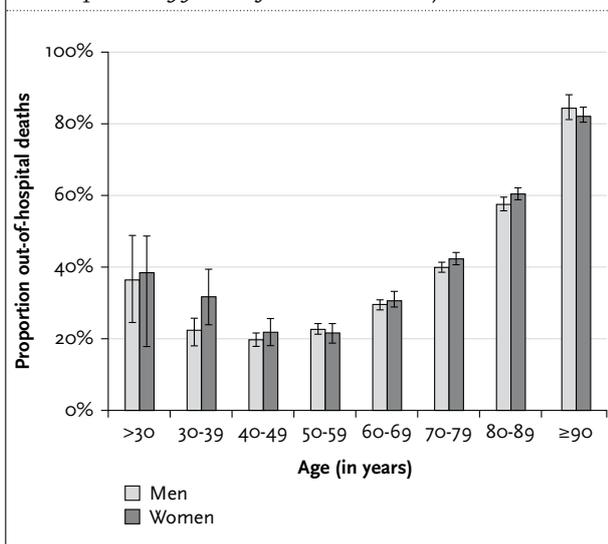
men compared with women were shown, as well as a large proportion of out-of-hospital deaths.

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.[18] This unfortunately was based on the principal diagnosis for all patients and all specialities. 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,

unfortunately. 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.[19] 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%.[19]

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,[20,21] 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.[22,23] 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

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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,[24] a population-based cohort study among 7983 men and women aged 55 years and over showed that 1.7% 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[25] 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.[26] If we had included silent AMIs, this probably would have yielded much higher estimates, as De Bruyne *et al.*[27] 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 subjects of the cohort that could not be traced back completely in the period 1995-2000, because they were not always unique on the linkage variables (approx. 6%) or they immigrated to the Netherlands (<1%) in this period. As previous admissions of these subjects could be missed, this might have led to a slight overestimation of the incidence. A last aspect of our study that needs to be considered is the exclusion of non-uniquely linked hospital admissions and deaths. If such exclusion is related to determinants of AMI risk, it might have affected the incidence estimates to some extent. A pilot study[14] suggested that non-uniqueness relates to large cities, foreign origin and age. The differences in these determinants between unique and non-unique persons were, however, relatively small.[19] Moreover, substantial bias in the incidence estimates is likely prevented by excluding non-unique persons in the PR in the estimates of person-years at risk.

The incidence of AMI has been addressed in a number of studies.[6-11,28,29] Yet, comparison between studies is difficult due to methodological differences (differences in data collection, registration methods, study population, case definition or research period). The different studies, however, were consistent with our finding of a higher incidence in men compared with women. In a Swedish national record linkage study, the incidence of first AMI (per 100,000) increased from 29 in men aged 25-44 years to 2322 in men aged 75-84 years and correspondingly in women from 8 to 1374. The male-to-female ratio decreased from 4.05 at age 45-54 years to 1.71 at age 75-84 years.[30] 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.*[31] 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.[32] 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.[29] 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.[32] 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.[33] These estimates are comparable with those in our study. In the FINAMI study,[34] 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.[35] 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.[36]

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.[37]

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.

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CASE REPORT

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 5×10^6 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
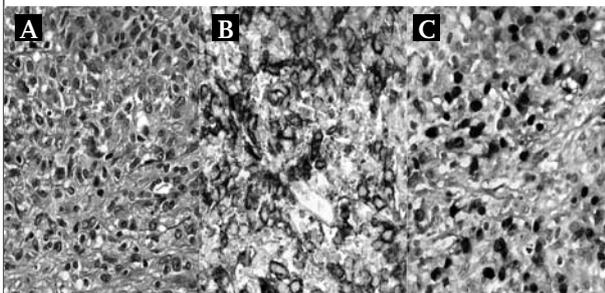

Figure 1. *Chest X-ray on presentation*

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

Table 1. *Laboratory results*

Test	Result	Test	Result
ESR	>120 mm/h	Base excess	-0.7 mmol/l
Haemoglobin	5.9 mmol/l	pO_2	7.9 kPa
Haematocrit	0.29 l/l	Urea	9.9 mmol/l
MCV	78 fL	Creatinine	81 μmol/l
Leucocytes	11.6 x 10^9/l	Sodium	134 mmol/l
Eosinophils	0.25 x 10^9/l	Potassium	4.0 mmol/l
Basophils	0.07 x 10^9/l	AP	193 U/l
Neutrophils	9.58 x 10^9/l	γGT	234 U/l
Lymphocytes	0.5 x 10^9/l	ASAT	39 U/l
Monocytes	1.18 x 10^9/l	ALAT	33 U/l
Platelets	415 x 10^9/l	LDH	395 U/l
pH	7.45	Albumin	29 g/l
pCO_2	4.3 kPa	Bilirubin	<5 μmol/l
HCO_3	22.4 mmol/l	ANCA	Negative

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

Table 2. *Chemotherapy regimen*

Cyclophosphamide	750 mg/m²	IV	Day 1
Doxorubicin	50 mg/m²	IV	Day 1
Vincristine	1.4 mg/m² (max 2.0 mg)	IV	Day 1
Prednisone	100 mg	PO	Day 1-5
Rituximab	375 mg/m²	IV	Day 3 (cycle 1-2)[*], day 1 (cycle 3-6)
G-CSF (pegfilgrastim)	6 mg	SC	Day 2

Cycle duration 14 days. Rituximab was given on day 3 during cycle 1 and 2 in this protocol in order to prevent tumour lysis syndrome. 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 (H&E); (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*
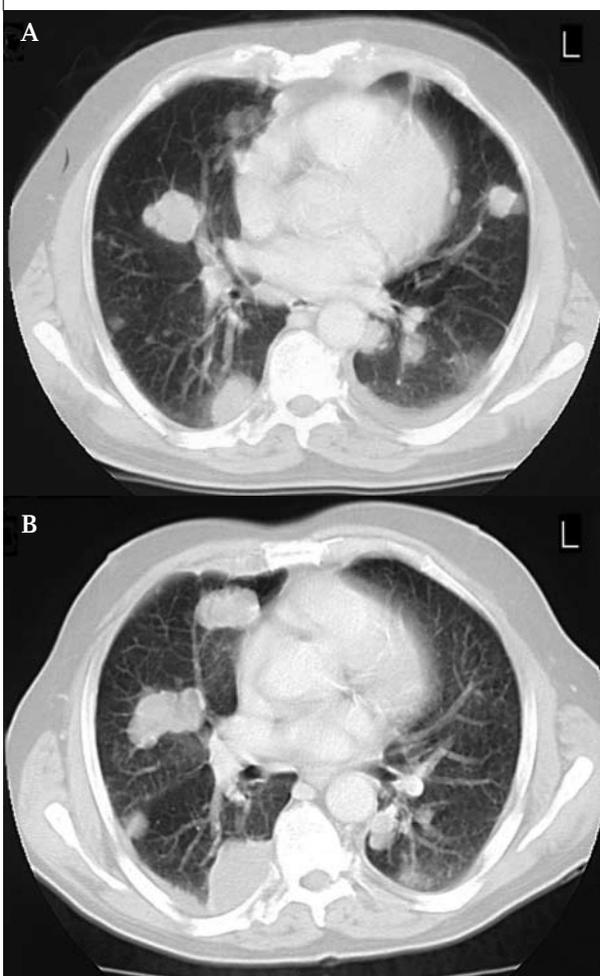

around the blood vessels. No yeast, fungi or acid-fast bacilli were detected but *in situ* hybridisation for Epstein-Barr virus (EBV) was strongly positive (*figure 2*). The diagnosis of lymphomatoid granulomatosis grade III was made. We treated him with intensified CHOP and rituximab every two weeks (*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.

INTRODUCTION

Lymphomatoid granulomatosis is a rare lymphopro-liferative disease. It was first described in 1972 by Liebow *et al.* during their studies of patients with Wegener's granulomatosis.[1] Lymphomatoid granulomatosis was

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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 effectivity 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.[2] Fauci *et al.* reported that three out of six lymphomatoid granulomatosis patients were anergic in response to common skin tests.[3] Due to clinical and histological similarities, it was suggested that lymphomatoid granulomatosis and polymorphic reticulosis (nasal and nasal type natural killer (NK) T cell lymphoma) were part of the same disease.[4] Together these diseases were called angiocentric immunoproliferative lesions (AIL).[5] A staging system for AIL, based on histological characteristics, was developed and proved to have prognostic value in small series of patients.[6] 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.[7] 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.[8] This was, however, not a widely held opinion. In their initial description Liebow *et al.* suggested a relationship between lymphomatoid granulomatosis and EBV infection.[1] This relationship was confirmed in 1990 when EBV DNA was found in tissue samples of 21 out of 29 patients with lymphomatoid granulomatosis.[9] Guinee *et al.* combined *in situ* hybridisation for EBV with immunohistochemistry in tissue samples from ten patients with lymphomatoid granulomatosis.[10] 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.[11]

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.[3,8,9,12,13] 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 erythematous 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.[8,3] 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 haemophagoytic 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,

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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.[3,11,12]

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.[8] 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.[14] 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.[12] Guinee *et al.* demonstrated a negative correlation between the amount of EBV-positive B cells and survival.[10] 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.[15] Response to treatment in patients with PTLD is accompanied by a prompt decline in viral copy number.[16,17] 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.[2,12] The largest series described is a retrospective analysis of different treatment strategies in 147 patients.[12] 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=13), 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).[3] 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.[18] 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.[11]

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

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(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.[19] 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.[20,21]

In 1986 Bernstein *et al*. described a 19-year-old patient with lymphomatoid granulomatosis with recurrent disease after COP chemotherapy.[22] 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.[23,24] 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,[25-27] one patient had a major response after four courses but died of haemoptysis[28] and two patients had progressive disease.[29,30] Two patients with lymphomatoid granulomatosis treated with CHOP in combination with rituximab have been described.[31,32] One patient was still in complete remission after 18 months of follow-up.[32] The other patient concomitantly received systemic and intrathecal methotrexate for CNS localisation.[31] 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.[33]

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.[34] 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.[11] 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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CASE REPORT

Watery diarrhoea: an unusual manifestation of breast cancer

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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

Table 1. *Laboratory values on admission*

|	Value	Normal range
Haemoglobin (mmol/l)	8.7	8.4-10.9
Thrombocytes (x 10^9/l)	362	150-400
Leucocytes (x 10^9/l)	8.3	3.5-11.0
Sodium (mmol/l)	138	137-145
Potassium (mmol/l)	2.7	3.6-5.0
Chloride (mmol/l)	108	97-107
Bicarbonate (mmol/l)	18	22-30
Urea (mmol/l)	3.3	2.5-7.0
Creatinine (μmol/l)	92	70-130
Glucose (mmol/l)	5.9	3.5-6.0
Lactate dehydrogenase (U/l)	349	200-450
Albumin (g/l)	38	35-50
Total protein (g/l)	69	60-80
C-reactive protein (mg/l)	<1	0-10
Thyroid-stimulating hormone (mU/l)	1.70	0.3-5.0

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

Figure 1. *Computer tomography of the thorax fused with somatostatin scintigraphy reveals a hot spot in the left thoracic wall suggesting a neuroendocrine tumour*

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

Figure 2. *(A) Tumour shows both solid and trabecular growth, and also forms glandular structures (H&E 5x); (B) Tumour cells are large with a rather monotonous enlarged nucleus with a prominent nucleolus. Mitotic figures are sparse (H&E 40x); (C) Tumour cells show neuron-specific enolase reactivity in the cytoplasm (40x); (D) Clusters of tumour cells show positivity for synaptophysin (40x)*

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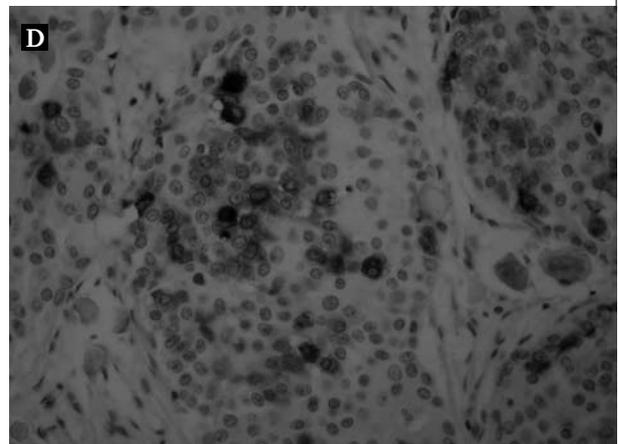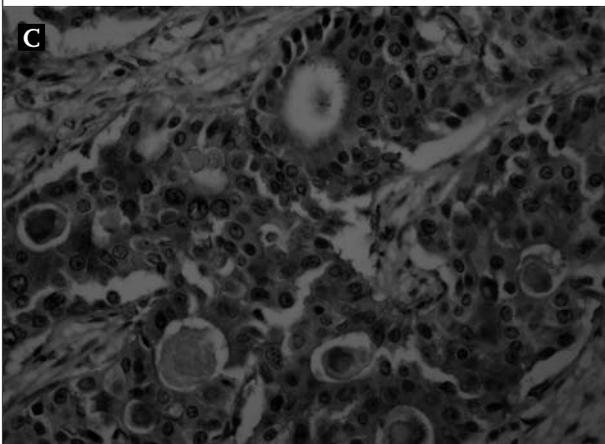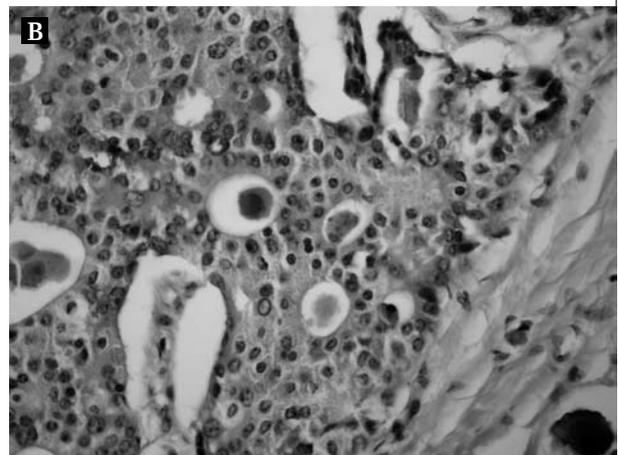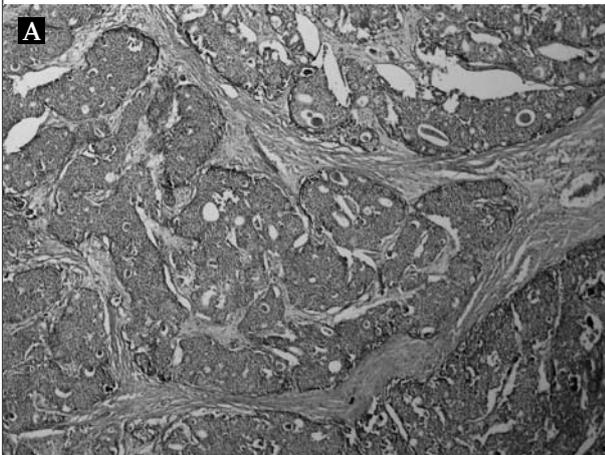
and the patient had an uneventful recovery. As an outpatient he remained asymptomatic during a follow-up of 12 months. Hormonal analysis, eight weeks after surgery, showed a normal level of PP (62 pmol/l). Serum gastrin level remained high at 997 ng/l.

DISCUSSION

Feyrter and Hartmann were the first to describe two patients with breast cancers with carcinoid growth patterns.[1] Neuroendocrine differentiation can be identified in up to 30% of breast cancers.[2] Neuron-specific enolase (NSE) is a well-known marker to demonstrate neuroendocrine differentiation and the same is true for chromogranin and synaptophysin.[2] 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).[3] 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).[2] 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,[4,5] while others only classify a tumour as neuroendocrine when the majority of tumour cells display neuroendocrine characteristics.[6] The described case showed activity of NSE in all tumour cells, while the positivity for synaptophysin was demonstrated in clusters of malignant cells.

The elevated levels of PP and gastrin supported the idea of a causal relationship between the diarrhoea and the tumour. Functionally active gastrointestinal neuroendocrine tumours have the ability to secrete multiple peptides into the plasma, thereby causing a chronic diarrhoea syndrome.[7,8] For many years it had been supposed that measurement of plasma peptide could be the way to diagnose such tumours with diarrhoea as first manifestation.[7,8] However, the diagnostic value of fasting plasma peptide concentrations to detect tumours in patients with chronic diarrhoea is questionable, as described by Schiller *et al.*[9] In their series of patients with chronic diarrhoea, none of whom had a neuroendocrine tumour, 45% showed elevated plasma peptide levels.

A serum gastrin level of more than 1000 ng/l is almost always due to Zollinger-Ellison syndrome.[10] Chromogranin A is another useful test in the diagnostic workup for suspected gastrinoma.[11] However, chromogranin A was not measured in the underlying case. The probability of Zollinger-Ellison syndrome was not supported by the findings on gastrointestinal endoscopy, lacking active ulcer disease and lacking prominent gastric folds.[12] Furthermore, high gastrin levels, in the absence of gastrinoma, can be ascribed to the chronic use of proton pump inhibitors (PPI), chronic diarrhoea and to gastric outlet obstruction,[10] as were present in the described case, although usually, with the long-term use of PPI, gastrin levels do not exceed 400 ng/l.[13] Finally, serum gastrin remained unchanged after the mastectomy and the chronic diarrhoea disappeared postoperatively. Therefore, an underlying gastrinoma was not a plausible explanation in this case. The highly elevated gastrin was most probably caused by PPI use combined with gastric outlet obstruction.[13]

Pancreatic polypeptide, besides chromogranin A, is considered to be a general marker for endocrine digestive tumours.[14] Also, a direct relationship between elevated PP due to PPoma and watery diarrhoea has been described.[15,16] However, in the underlying case the diarrhoea as such is the most probable explanation of the elevated serum PP level, supported by the failure to detect PP in the tumour by additional immunohistochemical examination and by the normalisation of serum PP postoperatively when the diarrhoea had disappeared.

Therefore, in the underlying case we were not able to determine which plasma peptide was responsible for the chronic diarrhoea. Nevertheless, the clinical picture and course strongly support the relationship between the tumour and chronic debilitating diarrhoea.

The present report supports the diarrhoeogenic potentials of neuroendocrine cells originating from a malignancy outside the gut or pancreas, more specifically from a male with breast cancer.

ACKNOWLEDGEMENTS

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

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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/nvkc)

Figure 1. *Diagnostic diagram for suspected iron accumulation*

Determine ferritin and transferrin saturation

Raised ferritin > reference lab for age/sex

Ferritin not raised

Exclude: infections, inflammations, secondary haemochromatosis

TS >0.45

TS ≤0.45

DNA diagnostics *HFE*

HH cannot explain current symptoms

C282Y/C282Y or C282Y/H63D

No C282Y/C282Y or C282Y/H63D

MRI: Iron too high

MRI: Iron normal

Diagnostics HH type 1*-4

HH cannot explain current symptoms

Ferritin ≤1000 µg/l

Ferritin >1000 µg/l

Liver biopsy (fibrosis/cirrhosis?)

Cirrhosis: α1- foetoprotein/ ultrasound scan and if necessary, liver MRI every six months

Phlebotomy until ferritin < 50 µg/l
Follow-up
Family screening

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)

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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.

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NOTE

The guideline development was initiated by the Netherlands Association of Interal 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, clinical chemists and clinical geneticists.

The working party consisted of the following people:

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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 hypoechogenic 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 ecchinococcus 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

Figure 2. *Abdominal CT scan performed after six days of antibiotics showing the disappearance of the abscess secondary to its fistulisation into the stomach*
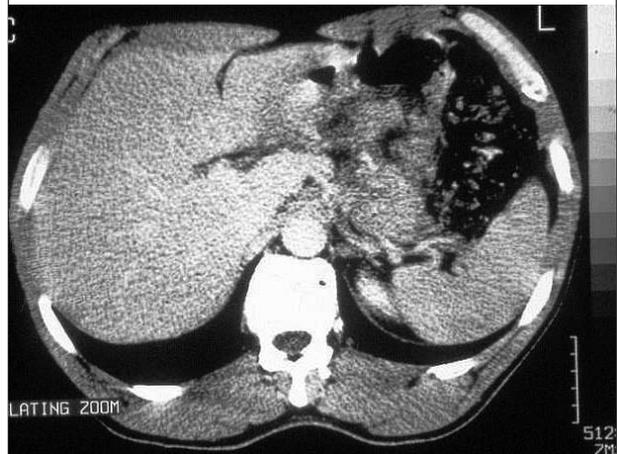
drained through a spontaneous fistula into the stomach (*figure 2*). The outcome was good and the patient was discharged.

CONCLUSION

PLA occurs with an incidence of 22 to 446/100,000 admissions.[1,2] Predisposing risk factors are diabetes mellitus, alcoholism, malignancies, immunodeficiency or liver transplantation. The mean age ranges from 50 to 60 years old,[1,2] with a male predominance. The main aetiology is cryptogenic, followed by biliary and inflammatory bowel disease.[3]

The three most observed clinical symptoms are fever, right hyponchondrial pain and nausea. A hepatomegaly is found in 25% of cases.[1,4] PLA is usually solitary and located in the right liver lobe.[1,5] CT scan with contrast media is the gold standard technique to visualise PLA, although ultrasonography is a reliable imaging procedure.[1,6] 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]

Figure 1. *Abdominal CT scan with contrast medium performed before the patient's admission showing a 5.5 cm abscess of segment II of the liver (black arrow), located in the front of the anterior face of the stomach*

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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MONTHLY NJM ONLINE HITLIST

The table lists online hits for all articles published in the September issue of the Netherlands Journal of Medicine, 2007 (available online on PubMed since 24 September 2007).

Article	Hits
EDITORIAL	
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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*

PATIENT WITH DIARRHOEA, ABDOMINAL PAIN AND WEIGHT LOSS

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.[1] 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

1. Buckley JA, Fishman EK. CT evaluation of small bowel neoplasms: spectrum of disease. RadioGraphics 1998; 18:379-92.

Figure 1. *Abdominal CT scan 1*

Figure 2. *Abdominal CT scan 2*

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?

Figure 1. *Fundoscopy*
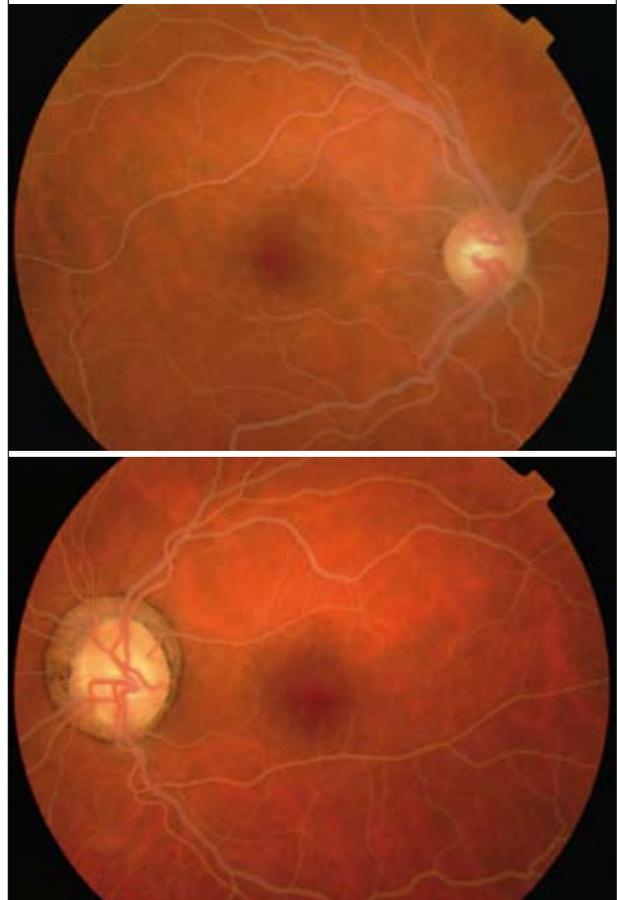
DIAGNOSIS

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.

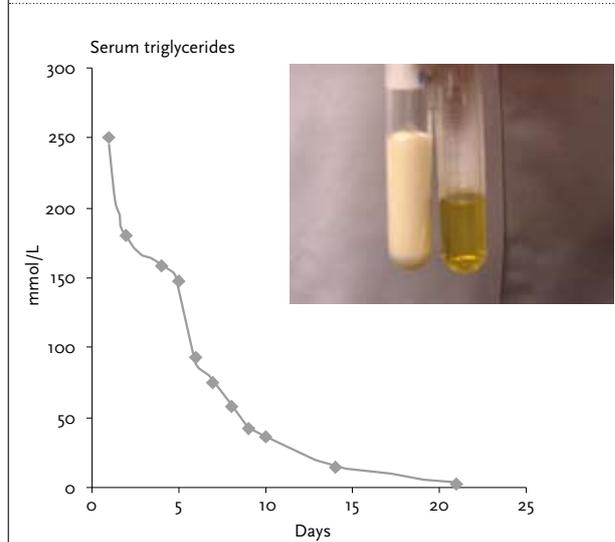**Figure 2.** *Lipaemic serum on admission and in the course of three weeks*

Serum triglycerides

Figure 3. *Disappearance of creamy appearance of retinal vessels after normalisation of serum triglycerides*

INFORMATION FOR AUTHORS

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.

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

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