

Review article

Screening for hemochromatosis

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Abstract

Background: Hereditary hemochromatosis is the most common autosomal recessive disorder in populations of northern European descent. **Issues:** Many experts consider hemochromatosis to be an almost ideal disease for population screening because it essentially fulfills almost all the criteria for screening proposed by the WHO. However, others disagree and suggest that more data are required particularly with regard to the natural history and penetrance of the disease. There is also disagreement about the best diagnostic/screening test for the disease and the performance of these tests in the context of screening. Other concerns are the variability and lack of standardization in screening test measurements, the selection of screening threshold values and the identification of false positive cases. The advent of a genetic test for the condition has brought other worries with regard to informed consent and the ethical, legal and social implications of screening particularly in relation to medical and general discrimination. Other important issues include compliance, cost effectiveness and the evidence that screening has lessened the burden of disease in the community. **Conclusions:** At the present time, we believe that further data regarding both the exact disease burden and the outcomes of screening studies particularly in the general community are required before widespread population screening is introduced. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Hemochromatosis; Screening; Transferrin saturation; Total iron-binding capacity; Unsaturated iron-binding capacity; *HFE* genotyping

1. Introduction

Hereditary hemochromatosis (HHC) is an autosomal recessive condition associated with an inappropriate increase in intestinal iron absorption. Iron

overload develops primarily because physiological mechanisms to eliminate excess iron are limited. Approximately 90% of excess iron is retained in the liver [1] and, therefore, the most common clinical manifestation of long-term iron overload is hepatic fibrosis or cirrhosis. The effects of iron overload are also seen in other organs such as the pancreas, skin, heart, joints and pituitary gland leading to the previous classical description of HHC as “bronzed diabetes” (skin pigmentation, diabetes and cirrhosis) [2]. Traditionally, HHC was viewed as a rare, inherited disorder primarily affecting older men, often present-

Abbreviations: HHC, hereditary hemochromatosis; *HFE*, hemochromatosis gene; SF, serum ferritin; TS, transferrin saturation; TIBC, total iron-binding capacity; UIBC, unsaturated/unbound iron-binding capacity; HIC, hepatic iron concentration.

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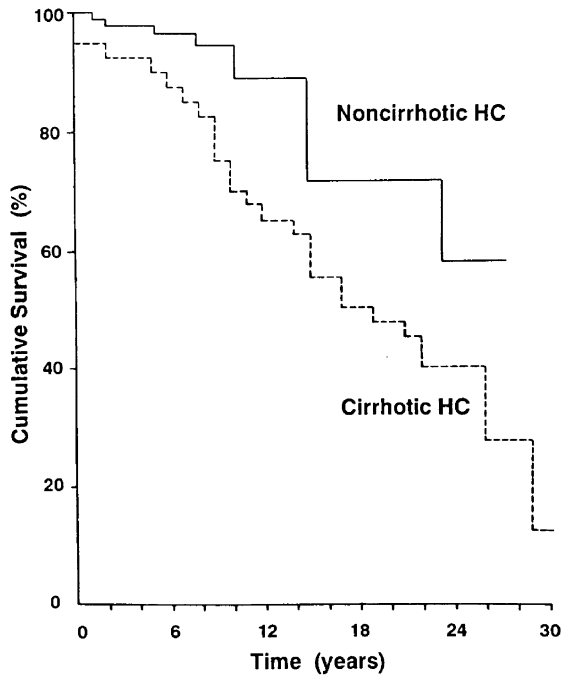


Fig. 1. Cumulative survival was significantly reduced in HHC patients with cirrhosis when compared with noncirrhotic patients (reproduced with permission from Niederau et al. [9]). The mean and distribution of age was similar in both groups.

ing with life-threatening complications such as diabetes mellitus, primary liver cancer or heart failure secondary to cardiomyopathy. However, the results of many recent studies from around the world have revealed that HHC is the most common autosomal recessive disorder in populations of northern European descent. The gene for HHC was discovered in 1996, and most patients are homozygous for the C282Y mutation in the *HFE* gene [3]. This gene is located on the short arm of chromosome 6. The C282Y genotype results from a single guanine (G)-to-adenine (A) point mutation resulting in a cysteine (C)-to-tyrosine (Y) substitution at position 282. Studies using genetic testing have estimated a prevalence of 1:188–1:327 in countries with a predominantly northern European origin [4] and the C282Y mutation has been found in > 90% of typical hemochromatosis patients in most of the populations studied [5]. However, rates of hospitalization, outpatient visits, and mortality due to HHC are much lower than expected given the prevalence of the condition [6,7],

suggesting that HHC is under-diagnosed and/or that the penetrance of the condition is low. Early manifestations in both men and women include nonspecific medical complaints such as fatigue, abdominal pain, sexual dysfunction or joint pain, and HHC is often overlooked as a potential underlying diagnosis [8]. The importance of a missed diagnosis is significant, as HHC is an eminently treatable disease. Patients who are diagnosed when they have end-organ damage such as cirrhosis have a significantly reduced survival compared with those without cirrhosis even if they are treated (Fig. 1). However, those who undergo and maintain iron depletion by phlebotomy and do not have cirrhosis, diabetes or cardiomyopathy at diagnosis have a normal life expectancy [9–12] (Fig. 2). Furthermore these studies have also shown that therapeutic phlebotomy often improves quality of life and may increase the longevity of patients who have these serious complications. Niederau et al. [9] reported that the life expectancy of HHC patients with cirrhosis who were

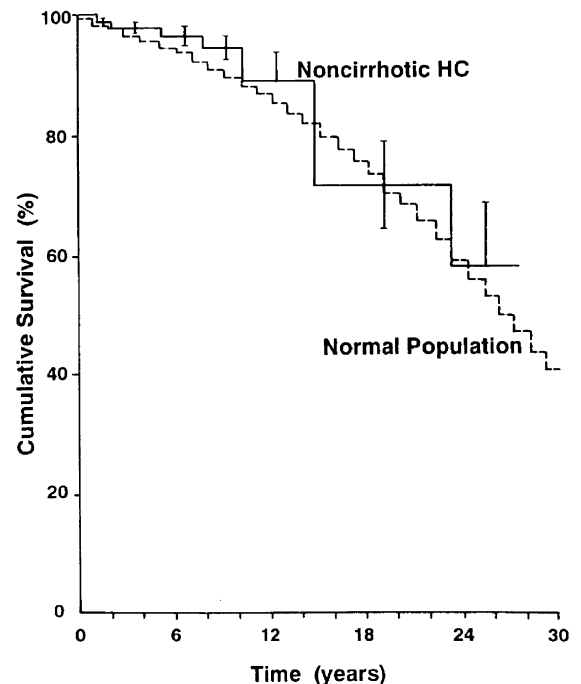


Fig. 2. Cumulative survival was similar for noncirrhotic HHC patients compared with that expected for an age- and sex-matched normal population (reproduced with permission from Niederau et al. [9]).

treated with phlebotomy was 10–20 years longer than that reported for other forms of cirrhosis, in particular that due to alcohol. One group have also found that with depletion of excess iron, varices were improved or completely reversed in 26% of patients with cirrhosis secondary to HHC. Of 22 patients with HHC in whom portal hypertension was unmodified or worsened, 16 had coexistent viral hepatitis [13].

HHC is considered by many experts to be an almost ideal disease for population screening in that it essentially fulfills almost all the criteria for screening, proposed by the WHO in 1968 [14]. It is an inherited disorder that has high prevalence in certain populations; it may have serious clinical manifestations including considerable morbidity causing premature death; there is a long latent period before complications occur; it can be identified by safe and reliable screening and diagnostic tests; and, after early diagnosis, can be treated effectively and inexpensively to prevent later complications [15].

However, other experts, particularly those specializing in public health medicine, disagree and suggest more data are required especially in the following

Table 1
Outstanding issues with regard to HHC screening and research

Major issues in the implementation of screening programs for HHC

- (1) Changes in the case definitions of hemochromatosis.
- (2) Selection of screening threshold values and identification of false positive cases.
- (3) Variability and lack of standardization in screening test measurements.
- (4) Physician education.
- (5) Informed consent and concerns about medical and general discrimination.
- (6) Patient compliance with screening and therapy.
- (7) Incidental detection of iron deficiency.

Research priorities in HHC

- (1) Characterization of the relationship between genotype and phenotype.
 - (2) Determination of the optimal approach to screening for iron overload.
 - (3) Analysis of the cost effectiveness of screening for iron overload.
 - (4) Assessment of the ethical, legal and social implications of screening for HHC.
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From McDonnell et al. [18] and Brittenham et al. [15].

areas: (1) the natural history of the disease, and (2) the penetrance of the C282Y *HFE* mutation—documenting the proportion of individuals with HHC who will develop serious clinical manifestations if the condition is not identified (i.e., the disease burden), before widespread general population screening is introduced [16,17]. Furthermore there is still disagreement about the best diagnostic test for HHC and evidence is still lacking about the performance of these tests in the context of screening.

The outstanding problems with regard to screening and research priorities in HHC have recently been addressed and these are listed in Table 1. We will discuss these important issues further and review the important studies pertinent to these issues that have been published recently.

2. Case definition

Previously, the definition of HHC was based on clinical and pathological findings of iron overload with the exclusion of causes of secondary iron overload and other primary liver disease. Commonly, patients would undergo a liver biopsy or quantitative phlebotomy if a liver biopsy was contraindicated. Diagnosis of iron overload due to HHC was made on evaluation of the liver biopsy if at least one of three of the following were present: grade III to IV iron stores on Perls stain, hepatic iron concentration $> 80 \mu\text{mol/g}$ dry weight, hepatic iron index score ≥ 1.9 , or, in the case of quantitative phlebotomy, $\geq 4 \text{ g}$ mobilizable iron was removed (16 phlebotomies, each removing 500 ml of blood [250 mg of iron per 500 ml]) before the development of iron limited erythropoiesis [8]. Recently, as a result of increased emphasis on early diagnosis, population-based screening and the identification of the C282Y mutation, it has been proposed that the definition of HHC be changed. However, a fundamental controversy in screening for hemochromatosis is whether the disease should be defined on the basis of genetic testing, independent of iron status (genotypic definition, i.e., homozygosity for C282Y mutation), or should be defined on the basis of the degree of iron overload in the absence of other known causes (phenotypic definition—based on combined clinical history and examination and laboratory assessment,

increased transferrin saturation, serum ferritin, parenchymal iron overload on liver biopsy, quantitative phlebotomy and pedigree studies identifying other family members with iron overload) [4]. The difficulties raised by exploring new case definitions in HHC screening programs include the following: comparisons between program findings and previous research must be made by using reported prevalence based on similar criteria (the presence or absence of iron overload and clinical symptoms). The penetrance of hemochromatosis is substantially below 100% and, thus, genetic tests and even biochemical criteria for the early diagnosis of HHC may not fully correlate with clinical outcomes. Participating physicians may be unfamiliar with these new criteria and moreover, the existing literature may contradict screening protocols and confuse patient care [18]. In view of these problems, an expert panel recently met to consider a new definition and, after much deliberation, reached a consensus on definition and classification [19]. The expert group definition of HHC is the following: hereditary hemochromatosis is an inherited disorder resulting from an inborn error of metabolism which leads to progressive iron loading of parenchymal cells in the liver, pancreas, and heart. In its fully developed stage, organ structure and function are impaired. The common form of this disease is due to homozygosity for the C282Y mutation in the *HFE* gene. Individuals detected as homozygous for the C282Y mutation but without iron overload are best characterized as such, i.e., as carrying the genetic mutation that may lead to hemochromatosis but not yet having the disease. There are four stages of the disorder:

1. Genetic predisposition, but no other abnormality.
2. Iron overload (approximately 2–5 g), but without symptoms.
3. Iron overload with early symptoms (lethargy, arthralgia), and
4. Iron overload with organ damage, especially liver cirrhosis.

In the same issue a separate expert panel emphasized that “the disease HHC is defined in terms of phenotype not genotype. Thus, the identification of any of the *HFE* mutations is, by itself, insufficient

for the diagnosis of HHC. Instead, the identification of the genetic abnormality provides evidence of susceptibility to developing the phenotype.”

The expert group also noted that there were other hereditary forms of hemochromatosis that may be secondary to other mutations in the *HFE* gene or other genes involved in iron metabolism. For example, a significant number of families in Italy who fulfill all the criteria for a clinical diagnosis of HHC are not homozygous for the C282Y mutation [20,21]. Piperno et al. [22] recently reported two novel nonsense mutations of the *HFE* gene in five unrelated Italian patients with hemochromatosis. Another Italian group found a homozygous nonsense mutation (Y250X) in the transferrin receptor-2 gene in six patients (from two unrelated families of Sicilian origin) with hemochromatosis [23]. It is likely that other genetic mutations affecting iron metabolism will be identified in the future and that some further modification to the definition of HHC will follow.

3. Screening methods

Screening strategies include phenotypic screening, phenotypic/genotypic screening and genotypic/phenotypic screening [4,19].

3.1. Phenotypic screening

Possible phenotypic methods of screening include serum iron, serum ferritin, transferrin saturation, total iron-binding capacity saturation, and unsaturated/unbound iron-binding capacity.

3.1.1. Serum iron

The potential advantages of serum iron are that it is inexpensive and its measurement can be automated. However, the disadvantages that preclude its use alone as a screening method are that it may not detect up to 90% of affected subjects [24] and concentrations are labile with a marked diurnal variation, and are affected by many other factors such as concomitant disease and diet [8].

3.1.2. Serum ferritin (SF)

The potential advantages of serum ferritin as a screening test for HHC are its moderate expense and its measurement is automated. Ferritin measurement

is a highly sensitive test for iron overload and so a normal concentration essentially rules it out.

However, its disadvantages are that its “normal” value rises with age and gender and it is elevated in many conditions other than iron overload such as inflammatory disorders, cytolysis, dysmetabolic syndrome or cancer. Only in the absence of these confounding conditions is the serum ferritin concentration an accurate reflection of both body iron stores and hepatic iron concentration.

3.1.3. *Transferrin saturation (TS) and total iron-binding capacity (TIBC) saturation*

TS (iron/transferrin) is regarded by many experts to be the gold standard phenotypic test for HHC and, therefore, the standard by which other tests are judged [25,26]. However, the terminology with regard to TS is imprecise and confusing [27]. The gold standard method of measuring TS requires colorimetric measurement of iron and immunological measurement of transferrin (either by nephelometry or immunoturbidometry) [27,29]. TS can then be calculated using the known molecular weight of transferrin.

The majority of previous epidemiological studies have not used this precisely standardized assay. Instead they measured total iron-binding capacity (TIBC = serum iron + unsaturated iron-binding capacity (UIBC) and used a calculated TIBC saturation (iron/TIBC) as a surrogate marker of TS. TIBC assays have been available for many years but many have not been as carefully standardized as have the direct transferrin assays. We and other groups have shown that bias is inherent in all TIBC assays and may be substantial [28,29]. Using reference material from the Center for Disease Control we validated our transferrin assay, and then compared the results with a commonly used TIBC assay (Roche Diagnostics) in 60 patients. Although the results from the two assays were highly correlated ($r^2 = 0.985$), there was a significant bias ($y = 1.15x$) [28]. The most important consequence of this bias was that a TIBC of 45% (demonstrated to identify 98% of persons with HHC [30]) equates to a TS saturation of approximately 52% with the Roche transferrin and iron assays. This is important, as the other controversial factor with regard to the use of TS in population screening studies is the selection of “cut off” or threshold values. These values are chosen to estab-

lish a screening threshold that would be sensitive enough to identify most cases of HHC without producing too many false positive results. However, the reported “cut off” values for TS in different studies have ranged from 45% to 70%. As indicated above, one possible reason for this variation may be the methodology used. Careful analysis of the literature reveals that studies using TIBC saturation as a surrogate for TS used cut off points greater than 45%, i.e., 58–70% [28]. However, those which used the “gold standard” method used a cut off of 45% [31,32]. Indeed McLaren et al. [30] using statistical mixture modeling on the data reported in the study of Leggett et al. [31], showed that a TS of 45% identified 98% of hemochromatosis homozygotes without misidentifying any normal individuals. In Olynyk et al.’s [32] study, the sensitivity was 94% with a cut off of 45%. Certainly a lower cut off has been suggested for women because it has been shown that at a relatively high TS such as 62% or more, 40% of putative female homozygotes do not screen positive for HHC compared to 100% of male homozygotes [33]. We and others [15,18,28,34] suggest that transferrin saturation test standards and quality control must be established for laboratories nationwide as was done for the national cholesterol awareness campaigns aimed at the reduction of cardiovascular disease. In addition, future studies should specify whether a TIBC or transferrin assay was used to determine the TS, and if a TIBC saturation was reported, whether it was calibrated against a true transferrin assay.

The potential advantages of TS are its moderate reagent costs and it is an excellent phenotypic marker of iron overload. The disadvantage is that the transferrin concentration is affected by other factors such as disease, age and hormones. It also has a diurnal variation such that the results of initial and repeated TS tests may vary by as much as 50% in the same person. Interestingly, however, Edwards et al. [35] have shown that there was no clear diurnal variation in TS in patients with HHC. TS may be artefactually changed by recent ingestion of iron or vitamins and any follow-up studies require the patient to be fasting. Even with these dietary restrictions, however, TS can still vary considerably in the same person. It has been demonstrated that having a repeat TS on a fasting sample decreased the number of false positive cases in a phenotypic screening strategy [35].

However, previous studies have shown that more than 20% of cases will not return for further investigation [18,36]. This is important when we consider the issue of patient compliance (see Section 5 below).

TS can also be decreased in inflammatory states in patients with and without HHC. Distant et al. [37] recently showed that median TS values among C282Y homozygotes were considerably lower when they were admitted with inflammatory disorders such as pneumonia compared with the convalescent state (27% vs. 71%). They found that C282Y homozygotes respond to inflammation by desaturation of transferrin and an increase in ferritin similar to normal individuals. Both TS and ferritin can also be affected by alcohol. Alcohol can cause increases in both TS and ferritin and with total abstinence their concentrations can return to normal.

3.1.4. Unbound / unsaturated iron-binding capacity (UIBC)

The potential advantages of UIBC are that it is a one-step automated and relatively inexpensive biochemical test. The UIBC is not a new assay and, as discussed above, has been used by many laboratories for their calculation of TS or as a surrogate marker of TS. It has been reported to identify 100% of iron overloaded patients and 95% of patients with normal iron stores [38]. It has previously been proposed as a suitable screening tool for HHC [25,39]. This view has been supported by two recent population studies using different patient groups in Canada and Australia. A population screening study in blood donors in Canada using UIBC to detect C282Y homozygotes found the UIBC outperformed the TS test with a higher sensitivity and fewer false positives [40]. However, this study has been criticized because the method used for measuring TS (i.e., TIBC saturation) was not the “gold standard” and blood donors are not an appropriate population for studying sensitivity and specificity data because therapeutic phlebotomy is the principal treatment modality for HCC. It is, therefore, questionable to extrapolate comparisons of UIBC and TIBC saturation in predicting homozygosity for C282Y from blood donors to the rest of the population [27].

A similar strategy using UIBC has been shown to be an effective screening method for the detection of C282Y homozygotes with some degree of iron over-

load [41]. A total of 5182 persons were screened with UIBC and where this was low (correlating approximately to a TS of > 40%), genotyping was performed. Nine homozygotes with increased TS were identified at a cost of approximately US\$1500 each. The study can be criticized, as genotyping was not performed on all subjects. However, the design of the study was not specifically to test the sensitivity and specificity of UIBC in identifying persons with HHC, but rather to test the economic efficiency of such a protocol. Considering the minimal additional cost of adding measurement of serum iron and calculating TIBC saturation, screening with UIBC alone does not appear to be desirable.

Because of its low cost, high sensitivity and acceptable specificity, TS, regardless of how it is calculated, is an appropriate initial screening test for HHC. The most troublesome aspect of this test, however, is its low positive predictive value [42], which is largely due to the low prevalence of HHC in the general population, and the presence of many other possible causes of iron overload (see Table 1 in [19]). These problems are compounded by the effects of biological variability and uncertainty about laboratory standardisation of TIBC and transferring assays [18,28]. The result is that hemochromatosis screening programs using these phenotypic markers will produce numerous false positive test results that must be differentiated from true cases of HHC by follow-up testing and careful evaluation [36]. It is of considerable importance to understand that identifying a possible secondary cause for iron overload should not stop a person being investigated for HHC. In our recent screening study we found that if we had excluded patients with apparent secondary causes of iron overload from subsequent evaluation for *HFE* genotyping we would have missed seven out of the nine C282Y homozygotes identified [41].

3.1.5. Incidental detection of iron deficiency

Another potential advantage of using phenotypic tests as the initial test in screening programs for HHC is that they will also detect iron deficiency as well as iron overload (in economic terms, a positive externality). In fact, previous screening programs using TS have picked up approximately 50 times more cases of iron deficiency than those with HHC [18]. The iron deficiency may be a result of serious

underlying pathology such as colorectal cancer, peptic ulcer disease and malabsorption. The high prevalence of iron deficiency means that an HHC screening program must include a strategy for follow-up of patients with low TS (< 15%). A full evaluation will obviously involve more time, personnel, and financial resources that will have to be taken into account in cost-effectiveness analyses.

Although their use in population screening studies looks promising, TS, TIBC saturation and UIBC need to be assessed further in more diverse populations. They must be evaluated in an appropriate spectrum of patients (like those in whom it would be used in practice). A consistent case definition also needs to be decided upon so that each potential test can be independently and blindly compared with a reference ('gold') standard and the reference standard applied regardless of the screening/diagnostic test result [43].

3.2. Genetic screening

An alternative to phenotypic screening is initial testing with genotyping for the C282Y mutation. The potential advantage of this is that it can be carried out at any age. The test could even be carried out at birth as part of neonatal screening for other disorders and pilot studies have already been conducted [44–46]. Another potential advantage of neonatal screening would be that parents of children with positive results could also be screened (i.e., "reverse" cascade screening) [45]. Allen and Williamson [47] believe that genetic population screening is the correct strategy and should be implemented.

The major disadvantages of genetic screening, are that both iron loaded and nonexpressing homozygotes would be detected and that genotyping is substantially more expensive than phenotyping. In addition, if neonatal screening was undertaken, mechanisms that provide long term follow up would need to be established. There is also the problem of families with HHC who do not carry the C282Y mutation. Although this is a particular problem in Italy [20,21], there are also families with hemochromatosis in other countries (e.g., in Africa) whose molecular basis still remains undefined [48]. False positive tests can also occur with genetic testing depending upon the techniques of molecular analy-

sis. Jeffrey et al. [49] recently reported that a G-to-A polymorphism at position 5569 in intron 4 of the *HFE* gene may interfere with the function of the antisense primer used for C282Y genotyping and, as a result, C282Y heterozygotes may have been misclassified as C282Y homozygotes. However, this finding is controversial, as others have disputed the significance of this polymorphism [50].

As discussed in detail below, there are significant issues regarding the penetrance of the C282Y mutation. For example, it has been reported that 30% of women homozygous for C282Y do not express the disease [51]. Detection of patients who do not and may never express the disease raises several psychosocial issues such as possible inappropriate discrimination by insurers and employers, difficult family dynamics, possibility of paternity disputes, labeling and stigmatization [52]. Because of these and other concerns (e.g., the optimal care of asymptomatic patients), widespread genetic testing/screening was recently rejected by two expert panels [5,19]. Moreover, although pilot studies of neonatal screening have already been carried out, other expert panels do not recommend testing in children [19] and the position of the US NIH-DOE Task Force on Genetic Testing is: "Genetic testing of children for adult onset disease should not be undertaken unless medical benefit will accrue to the child and this benefit would be lost by waiting until the child has reached adulthood" [53].

A second mutation in the *HFE* gene, H63D, has been even more difficult to implicate in the pathogenesis of HHC. Some compound heterozygotes (C282Y/H63D) have been found to have mild to moderate iron overload often in association with other risk factors such as alcohol, hepatitis C and steatosis/steatohepatitis. It has been estimated that only 1.5% of compound heterozygotes will develop significant iron overload [54]. Similar findings have been reported by another group [55]. H63D homozygotes are common in the general population and have only rarely been found to have iron overload. Moirand et al. [55] suggest that the H63D mutation alone is not sufficient to cause significant iron overload but is a cofactor of phenotypic expression in other situations susceptible to being associated with iron-overload, such as alcoholism, metabolic disorders, cirrhosis or C282Y heterozygosity.

A third mutation resulting in a serine-to-cysteine substitution (S65C) localized to exon 2 close to the H63D mutation has been implicated in mild forms of HHC in patients without the C282Y and H63D mutations [56]. The significance of this mutation requires further examination.

4. Penetrance and disease burden—relationship between genotype and phenotype

A common concern with regard to introducing widespread population screening programs for HHC and another factor which will affect the cost effectiveness of such programs is the relative lack of data on the penetrance and, hence, the disease burden of HHC. The prevalence of clinical disease due to HHC is uncertain. Based on the estimated prevalence of homozygous HHC, either from studies using a case definition of homozygosity for C282Y (prevalence 1:188–327) [4] or those using iron overload (prevalence 1:200–300) [31,57], we would expect a more substantial disease burden from HHC in terms of both morbidity and mortality. As discussed previously, this does not appear to be the case and this may be due to either underdiagnosis or incomplete penetrance.

A major concern about the use of genetic testing in screening for HHC is the variable phenotypic expression of the disease. Indeed, the most important research aim identified by a Working Group on Research Priorities in HHC was better characterization of the natural history of the relationship between genotype and phenotypic expression in HHC and other iron overload disorders with respect to age, sex, ethnicity and environmental factors [15]. On review of recent studies, it appears that there are three different groups of patients with respect to phenotypic expression of the C282Y mutation:

1. C282Y homozygotes with severe iron overload and end-organ damage/or potential end-organ damage.
2. C282Y homozygotes with abnormal iron indices, suggesting a disposition to iron overload, but no end-organ damage clinically apparent and probably unlikely.

3. C282Y homozygotes but with essentially normal iron indices.

Studies of the clinical course and penetrance of HHC are often confounded by ascertainment bias: case series identified in referral centers and studies of relatives of highly expressing probands are likely to overestimate case severity, whereas studies of blood donors (which exclude persons with clinical or laboratory abnormalities and include “treated” cases) underestimate case severity [19].

In one preliminary study, before systematic screening for HHC, David et al. [46] picked up 18 (6 men, 12 women) C282Y homozygotes among the first 3017 subjects tested (0.6%). All of the men had increased TS (range 50–92%) but only six of the 12 women (range 49–98%). The others (i.e., 50%) of the women had no phenotypic expression of the disease in the absence of pathological blood loss. They also found that phenotypic expression was not related to age in men aged 25–40 years and women aged 35–50 years.

In a study of families of probands with HHC, Crawford et al. [51] found that 6.7% of men and 32.7% of women homozygous for the C282Y mutation did not demonstrate iron overload or meet the previously established clinical and biochemical diagnostic criteria for HHC. Most of the males, however, were young (23, 24, 26, 26 and 47 years) and 12/17 females were < 45 years (i.e., potential child bearing age) and two of the remaining five had other possible explanations for non-expression: one had Crohn’s disease and one had had 14 children. All males homozygous for the C282Y mutation had TS > 45%. The serum ferritin concentrations of these subjects were variable (165–1240 µg/l). Six of 17 females (35%) had TS < 45% and only 1/17(6%) had a ferritin > 200 µg/l (414).

In four recent screening studies for HHC, the proportion of C282Y homozygotes with a normal ferritin was 25% [32], 40% [58], 50% [59] and 80% [40]. However, the study by Adams et al. [40] was on blood donors. Thus, these persons may have already been treated and their ferritin and TS concentrations may not reflect the severity of the original expression of the disease.

Two more recent reports also suggest only mild to moderate biochemical expression in C282Y homozy-

gotes in Norway and the US. [37,60]. Distante et al. [37] looked at the prevalence and phenotypic expression of C282Y homozygotes among a hospitalised population in a high prevalence area of hemochromatosis in Norway. Of the 13 new cases detected, only one had cirrhosis and hepatocellular carcinoma. The median TS and ferritin in the remaining patients were 71% and 219 $\mu\text{g}/\text{l}$, respectively. Three patients had liver biopsies which showed iron staining (Perl's Stain) with a typical periportal pattern of grade 2+ but no signs of fibrosis. Hepatic iron indices were 1.8, 1.9 and 2.2. This was despite a median age of 73 years. Beutler et al. [60] detected 33 untreated C282Y homozygotes among 10,200 patients attending a health appraisal clinic in San Diego. The mean (range) TS and ferritin concentrations among the females were 49% (40–58%) and 146 $\mu\text{g}/\text{l}$ (90–237), respectively, and among the males were 54% (44–64%) and 314 $\mu\text{g}/\text{l}$ (165–598), respectively. Five of these 33 patients were frequent blood donors. All the homozygotes detected were clinically well. The authors emphasized that the apparent low expression of the homozygous C282Y mutation was not a function of the age of the population, since their patients were older than those in the study of Olynyk described below.

In the population-based study in Busselton, Australia, Olynyk et al. [32] found 16/3011 (0.5%) were homozygous for the C282Y. TS was > 45% in 15 of these 16 individuals and in the remaining patient it was 43%. Four of the 16 had previously been given a diagnosis of HHC. Seven of the 12 new cases (five males and two females) had increased ferritin concentrations (510–1677 $\mu\text{g}/\text{l}$). Six of the seven underwent liver biopsies. Two male patients aged 42 and 74 years had fibrosis and the male with the highest ferritin had cirrhosis. This patient had a previous history of excessive alcohol consumption. Eight of the 16 homozygous subjects (50%) had clinical findings that were consistent with HHC such as hepatomegaly, skin pigmentation and arthritis, and all were > 30 years. 30% of the homozygotes had no clinical suspicion of disease.

Willis et al. [61] examined the issue of penetrance further using a retrospective population-based analysis in a stable population in the east of England. They examined the frequency of homozygosity for the C282Y mutation in archived biopsy specimens

from patients with liver cancer and cirrhosis and compared this with the frequency in a normal population. They concluded that a diagnosis of liver cancer or cirrhosis is rare in the lifetime of individuals from the population who are homozygous for the C282Y mutation—just 2.5% (upper 95% CI 8%). Moreover, in this population, 98.6% of C282Y homozygotes were untreated.

The findings of Willis et al. is consistent with a low frequency of deaths attributed to hemochromatosis in the US. [7]. Willis et al. [62] have also reported the observation that C282Y homozygous men can survive to extreme old age without treatment. They looked at a sample of 600 patients > 70 years and found a prevalence of 1:150 for C282Y homozygotes. This suggests that homozygotes are not underrepresented in an elderly population because of death from life-threatening complications. The particularly low penetrance in the British population may be unusual and needs to be studied in other populations. Willis et al. [61] also suggested that it is also important to determine whether the C282Y homozygotes in their population who do not develop HHC-related liver disease do not accumulate large iron stores.

Cogswell et al. [34] recently reviewed studies looking at the estimated prevalence of elevated iron status and iron overload due to HHC among patients with chronic diseases. However, 14 of the 15 quoted studies lacked appropriate comparison groups (e.g., matched for age and sex), which hampered a reliable estimation of the proportion of clinical disease resulting from HHC. Screening studies have not compared the prevalence of clinical findings in persons with HHC and those without. Many clinical findings associated with HHC such as abdominal pain, fatigue, arthritis and diabetes are also common in patients without HHC.

As discussed previously, analysis of factors affecting phenotypic expression was stated to be an important research aim identified by the Working Group on Research Priorities in HHC [15]. Such factors are likely to be both genetic and environmental. Promoter regions upstream from the *HFE* gene or other genes not linked to *HFE*, may affect iron absorption, the degree of iron storage [63] and tolerance of iron overload in tissues [64]. Further evidence that additional modifying genes influence the

expression of the *HFE* gene has recently been reported by Whiting et al. [65]. It is likely that other mutations, both in modifying genes linked to the *HFE* gene and in other genes involved in iron absorption/transportation (e.g., *DMT1*, *Hephaestin*, *IREG1* and transferrin receptor), will be discovered in the future, which will explain, in part, the differences in phenotypic expression both in the population as a whole and within individual families [66]. Environmental factors such as diet (in particular intake of red meat), nutritional supplements (especially those containing vitamin C and iron), alcohol intake and viral hepatitis are also likely to be important. For example, differences in the consumption of meat in Australia and the UK [67,68], may help explain the apparent disparity in disease expression between these countries [32,61,62]. Alcohol in particular is a significant risk factor for the development of cirrhosis in patients with HHC [69]. In nonexpressing homozygotes (especially males), it is important to consider the possibility of a false positive genetic test (as discussed above in Section 3.2) or pathological blood loss (e.g., due to peptic ulcer disease, celiac disease or bowel cancer).

Further population studies are required to examine the long-term outcome of the different groups of C282Y homozygous patients particularly those in group 3 above (i.e., C282Y homozygotes with essentially normal iron indices). These are commonly premenopausal women who are protected from the clinical effects of HHC because of menstruation and pregnancy. What happens to their iron indices when they become postmenopausal? Typically, a woman in the western world will live for at least 25 years after the menopause and potentially there will be ample time for significant disease to develop resulting in a reduced life span. Another key question with respect to the decision about whether population screening should be implemented for HHC is what proportion of affected individuals will progress from biochemical evidence of iron overload to serious clinical manifestations? Such information would conventionally be obtained from inception cohort studies. However, the very long evolution of HHC precludes the acquisition of these data in a reasonable time period. An alternative method, such as cross-sectional sampling might be used instead [19]. However, early data have been reported by Olynyk

et al. [32] from their population-based cohort study in Busselton Australia. Over a 4-year follow-up period, six of the seven newly diagnosed C282Y patients who had an increased ferritin at diagnosis had a further increase of between 9% and 40%. The serum ferritin decreased in one patient from 564 to 526 $\mu\text{g/l}$. Of the five newly diagnosed patients with a normal ferritin one patient went from a normal concentration (145 $\mu\text{g/l}$) to abnormal (805 $\mu\text{g/l}$) but the concentrations remained normal in the other four. These were all females, aged 30, 41, 43 and 45 years. Of the nonexpressing cases from Adams' pedigree studies followed up for several years (mean 3.5 years), only one case had developed an elevated ferritin requiring venesection [70].

5. Cost-effectiveness of screening for HHC

Every health system in the Western world is under financial pressure as the demands for more sophisticated treatments mount. One consequence of this is that no proposed screening program will be given credence or supported unless it can be demonstrated to be both effective and relatively inexpensive. A major problem with assessing the cost-effectiveness of possible protocols for screening for hemochromatosis is that costs and charges are confused and frequently used interchangeably. For example, the charge for C282Y genotyping is variously quoted as being US\$125–175 [71] and US\$8 [72]. Clearly, such differences need to be resolved before an assessment of true costs can be made. The cost to a laboratory may be substantial if only a small number of samples are tested using a particular assay, but these costs may fall dramatically if large numbers of samples are tested, as is likely to be the case if a formal screening program is introduced. Thus, the most important initial consideration should be what the laboratory costs are per billable test, rather than the billable charge for the test. However, even despite using a very high quoted charge for C282Y genotyping (US\$173), data for HHC screening presented by Adams and Valberg [71] would appear cost-effective compared with acceptable guidelines [73], even if only 20% of patients develop life-threatening illness.

When compared with other screening programs such as those for breast cancer by mammography,

colon cancer by hemocult testing or colonoscopy and esophageal cancer with annual endoscopy, current estimates for the cost of HHC screening programs compare very favorably [41,74]. For example, Hickman et al. [41] using UIBC and then genotyping for *HFE* mutations reported the average cost per each HHC case detected was Australian \$2270 (US\$1500). To save 1 year of life by (1) mammographic screening for breast cancer in women > 50 years and (2) screening for colorectal cancer using hemocult testing of faeces costs \$21,400 [75] and \$20,000–30,000 [76], respectively.

There are still several points that must be clarified in a discussion of costs. First, it should be pointed out that the costs of both screening and treatment must be considered if the purpose of screening is to identify and treat affected individuals. Second, the difference between costs and charges must be clearly stated. Third, specific outcome measures should be assessed. Perhaps the most important of these is that blood taken from patients during treatment for HHC can now (in several countries) be used for blood donation (another positive externality), and this may reduce the cost of treatment to zero. The extra blood available would be very useful given the perpetual demands on blood banks for a limited supply of blood. This could prove to be especially valuable in Australia given the recent ruling that any Australian who spent more than 6 months in Britain during the period 1980–1996 will not be eligible to donate blood because of the potential risk of transmitting bovine spongiform encephalopathy (BSE). Estimates suggest that this will reduce the donor pool by 6%. In other countries, such as the US, blood from HHC patients is discarded. This increases the cost to patients, who may be charged for the procedure, and may, therefore, have an adverse effect on compliance. An expert panel recently stated that “HHC is not in itself a contraindication to using blood for transfusion or conversion to other products obtained from donated blood, and recommends its use in this way” [19].

6. Compliance

Other important factors that will affect both the success and cost effectiveness of a screening pro-

gram are enrollment rates and compliance. Enrollment and compliance rates have varied widely in previously reported screening studies for HHC. Adams et al. [40] reported a 97% enrollment rate in voluntary blood donors. They suggested that this was likely related to the fact that they were already undergoing venipuncture and have a history of altruistic behavior. In contrast, in a study of phenotypic and DNA testing among employees of health maintenance organizations in Springfield, MO, McDonnell et al. [59] reported an overall participation rate of just 28% (1653 of 6000) and 83% of those who participated were women. A similarly low enrollment rate of 30.3% was reported by Burt et al. [58] and Beutler et al. [60] recently reported that only 39% of patients attending a health appraisal clinic who were asked to take part in a study of DNA examination and phenotypic tests for hemochromatosis agreed to participate in the project.

McDonnell et al. [18] have reported high rates of compliance with phlebotomy therapy (70–84%). They also point out that although these figures may seem low considering the benefits of treatment described previously, they compare favorably with rates of compliance with other medical treatments, such as taking medication for hypertension, making dietary changes for obesity, or follow up of positive fecal occult blood. The same authors suggested that in all screening programs, the major variable influencing the enrollment rate and compliance with follow-up procedures was the concentration of interest and enthusiasm of primary care physician's [18].

7. Informed consent, ethical, legal and social implications of screening for HHC

Screening programs for HHC are receiving heightened ethical and legal scrutiny. This is mainly because hemochromatosis is a genetic condition. Furthermore, although both the TS and recently described genetic test can result in the diagnosis of HHC, human subject institutional review boards consider the gene test to be more sensitive and potentially more damaging. Previous screening programs, even those involving genetic testing, have not required written consent from the participants. Many have concerns about this type of testing both on

ethical and legal standpoint. However, it must be borne in mind that widespread screening and routine testing for other disorders considered to have a considerable genetic influence (e.g., hyperlipidaemia [77] and diabetes) continue to be carried out on a day-to-day basis worldwide without informed consent. Obtaining informed consent for genetic testing could also have a deleterious effect on enrollment and compliance in screening programs [18]. Detection of patients who do not and may never express the disease has far-reaching ethical, psychosocial and possibly legal implications such as possible inappropriate discrimination by insurers and employers [52]. For example, we have recently seen examples of insurance policies being refused or higher premiums being charged due to the results of genetic tests [78]. Some countries now have laws preventing genetic discrimination [79] and some insurance companies are beginning to recognize that early diagnosis and treatment of HHC can lead to normal life expectancy and would be of benefit to them rather than a disqualification. Preliminary studies on the psychosocial effects of genetic testing for hemochromatosis have so far not demonstrated any adverse effects [80] but further in-depth studies are required to examine this important issue further.

It should also be recognized that not all genetic conditions could and should be considered and handled in the same manner. For example, HHC can effectively be cured (provided end-organ damage has not already occurred) by simple phlebotomy, thus, making it a totally different condition to consider compared to, for example, Huntington's disease.

8. Which populations and at what age should be screened for HHC?

Effective screening must target those populations where the yield is likely to be the highest. Population data demonstrate that HHC is a European (particularly Celtic) disease. Thus, by definition, screening should be targeted to those populations around the world with predominantly northern European ancestry. The goal of screening is to detect homozygotes requiring venesection and ideally should take place as near as possible to the expected time of onset of clinical problems to minimize the duration of treat-

ment and follow up needed. It is obviously desirable to screen before the onset of end-organ damage. Phenotypic screening strategies, depend on HHC patients demonstrating increased transferrin saturation. This usually occurs in young adults older than age 20 years but can occur earlier. Onset of liver damage is uncommon before the age of 40 years. It is particularly important to diagnose HHC before the development of cirrhosis or diabetes as a normal life-span is the outcome if patients are treated with regular phlebotomy. In studies from France and the US, cirrhosis has not been observed in nonalcoholic patients under the age of 40 years without hepatitis C infection [81,82]. In a recent meta-analysis of screening and intervention trials Bradley et al. [42] found 7/28 (25%) male patients homozygous for the C282Y mutation had liver fibrosis. Two of these patients were < 40 years (one at 24 years and the other at 39 years). The other five were > 50 years. Interestingly, six of these seven patients had no signs or symptoms of the disease. Three of nine (30%) homozygous women had fibrosis (aged 46, 47 and 76 years). Eight of 28 males (29%) had signs or symptoms of HHC—cardiomyopathy, diabetes, hypogonadism, hepatomegaly or skin pigmentation. Three of nine (30%) females had signs and symptoms of HHC. Assessment of morbidity in terms of symptoms alone is very difficult and open to bias as many of the early symptoms of HHC—fatigue, abdominal pain and arthropathy—are nonspecific and very common in the normal population. Bradley et al. [83] also examined the morbidity for undiagnosed HCC in previously unidentified homozygous C282Y siblings of affected index cases. Under the age of 40 years, 55% of male and 43% of female homozygote siblings had one or more clinical manifestations of morbidity that could possibly have been attributed to HHC (such as abdominal pain, fatigue, arthropathy). Over the age of 40 years, 73% of men and 44% of women had morbidity. Studies such as these, however, are subject to ascertainment bias. Homozygotes identified because of the clinical sequelae of iron-overload all have HHC disease-related conditions, whereas screening of healthy subjects generally uncovers few clinically affected homozygotes [84]. Bulaj et al. [84] attempted to overcome the problem of ascertainment bias by studying homozygotes who had not been preselected for illness or good health.

To achieve this they looked at the homozygous relatives of probands identified because they presented with signs or symptoms of hemochromatosis (i.e., clinically affected) and probands identified on the basis of findings of elevated TS values during HHC screening or a routine health maintenance examinations. In this study, 214 homozygous relatives of 291 homozygous probands were identified. Of the 113 men in this group (mean age 41 years), 96 (85%) had iron overload and 43 (38%) had at least one HHC disease-related condition (cirrhosis, fibrosis, elevated aminotransferases, or hemochromatotic arthropathy). Of the 50 men aged between 21 and 40 years, 15 (30%) had at least one HHC disease-related condition. Of 101 female homozygous relatives (mean age 44 years), 69 (68%) had iron overload and 10 (10%) had at least one HHC disease-related condition. Among the 50 women aged between 21 and 50 years, only two (4%) had a HHC disease-related condition. Their minimum estimate of the incidence of HHC disease-related conditions among homozygous relatives of probands with elevated TS was 29% for men > 40 years and 11% for women > 50 years. They suggest that this is a reasonable estimate of overall disease incidence in homozygotes in the white population. One problem with this study, however, is that the threshold value for TS was set at 62% for both men and women. In the women in particular, this may have identified a subgroup of homozygous probands with a more highly penetrating phenotype.

These studies add further weight to the viewpoint that screening before the age of 40 is important. We and others [19,85] believe that 30 years is probably the optimum age for screening. One potential problem with such a strategy, however, is that it would not detect the nonexpressing premenopausal group.

9. Possible screening and management strategies

There are many possible screening strategies which could be followed. Currently the consensus from many expert panels is that phenotypic screening (preferably in young adults) in populations of predominantly European ancestry is probably desirable followed by genetic testing [5,19]. A possible screening and management strategy for HHC is shown in Fig. 3.

9.1. Selected population for screening

Previous screening studies for HHC have looked at a variety of different population groups e.g., employees of large corporate institutions [57,59], blood donors [33,40], hospital patients [37,41] and defined geographic areas such as Bussleton in Australia [32]. If widespread population screening is not introduced in the future, perhaps the most practical solution would be to perform serum iron studies when assessing young (< 40 years) ambulatory primary care populations.

9.2. Role of liver biopsy in the management of HHC

Genetic testing for the C282Y mutation has replaced the need for liver biopsy in many cases, particularly in young patients without liver dysfunction. This is very important from the standpoint of the implementation of population screening because the requirement for a liver biopsy has previously been a major impediment. Guyader et al. [81] investigated noninvasive predictors of liver fibrosis in C282Y homozygotes. They found that patients with a normal aspartate aminotransferase, no hepatomegaly, and a serum ferritin of < 1000 $\mu\text{g}/\text{l}$ have a very low probability of severe liver fibrosis. A similar study was carried out in Brisbane and showed similar findings, but in addition, concluded that a significant proportion of patients with alcohol intake > 60 g/day and > 45 years, had severe liver fibrosis or cirrhosis. They concluded that liver biopsy need not be performed if the serum ferritin concentration is < 1000 $\mu\text{g}/\text{l}$ and alcohol intake < 60 g/day [85]. Both studies concluded that a serum ferritin concentration > 1000 $\mu\text{g}/\text{l}$ was the strongest independent predictor of fibrosis of the liver. In Bradley et al's meta-analysis [42], however, 3/7 male and 2/4 female homozygotes with fibrosis had serum ferritins < 1000 $\mu\text{g}/\text{l}$ and no hepatomegaly, but no information on liver enzymes was given. The degree of fibrosis was also not stated.

There will still be a role for liver biopsy to assess the severity of liver disease (and, therefore, the prognosis and risk of hepatocellular carcinoma) in patients with liver dysfunction who have HFE mutations and as a diagnostic method in patients with iron overload without HFE mutations.

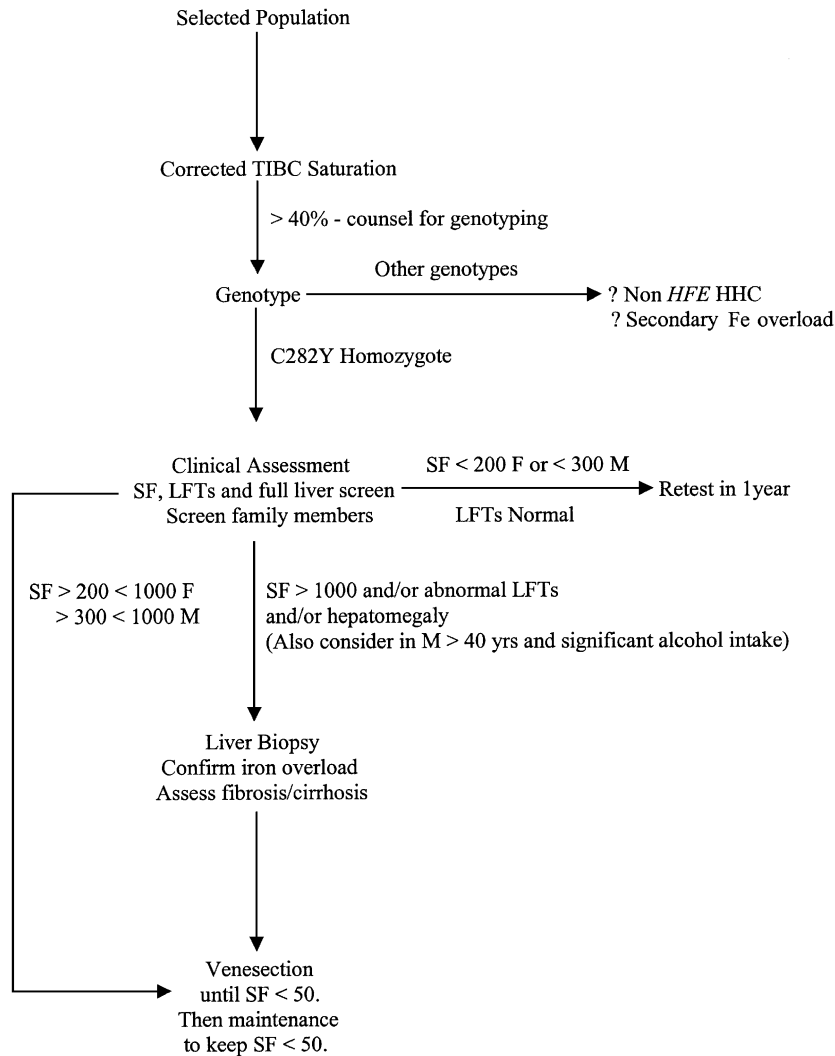


Fig. 3. A possible screening and management strategy for HHC. TIBC, total iron binding capacity; SF, serum ferritin ($\mu\text{g}/\text{l}$); LFTs, liver function tests; F, female; M, male. Maintenance therapy usually involves two to four venesections per year.

9.3. Venesection / phlebotomy therapy

The serum ferritin threshold concentrations (200 $\mu\text{g}/\text{l}$ in females and 300 $\mu\text{g}/\text{l}$ in males) we chose to commence therapeutic phlebotomy therapy in asymptomatic patients were those proposed by the Hemochromatosis Management Working Group [1]. This is still a controversial area because the natural history of asymptomatic hemochromatosis patients has not been clearly established. There is still uncer-

tainty as to whether these patients, if left untreated, would progress to life-threatening complications.

There is as yet little evidence of long-term benefit of therapeutic phlebotomy in the subgroup of patients who are asymptomatic, have normal LFTs, no hepatomegaly, and have an increased ferritin for age and sex (but $< 1000 \mu\text{g}/\text{l}$). However, Wojcik et al. [86] recently reported the long-term follow up of their cohort of asymptomatic C282Y hemochromatosis patients treated by venesection therapy. There

were no deaths or life-threatening complications and the long-term survival of these patients did not differ from an age- and sex-matched sample from the general population. For ethical reasons, it is very unlikely that long-term prospective randomized controlled trials comparing venesection with no treatment will be performed. It would be interesting, however, to compare this group with the majority of patients in the east of England and Jersey [61,87] who are not currently undergoing venesection.

9.4. Screening family members

Screening for HHC in parents, siblings, and children of affected patients (cascade testing) is an important consideration. El-Serag et al. [88] examined the cost-effectiveness of various screening strategies for HHC in siblings and children of affected patients. *HFE* gene testing of the proband followed by gene testing of the spouse was the most cost-effective strategy for screening two or more children. For example, screening two children was associated with an incremental cost-effectiveness ratio (i.e., cost per patient compared with no screening) of US\$3665 per additional life-year saved compared with US\$7934 for screening using serum iron studies. For siblings, compared with no screening, all screening strategies were dominant: that is, they cost less and yielded greater benefit than no screening. Strategies using *HFE* gene testing were less costly than serum iron studies [88].

10. What evidence is there that screening for HHC has lessened the burden of disease on the community

Two studies have suggested that early diagnosis and treatment of patients identified either by screening family members of affected individuals [89] or by screening laboratory test results [90] reduces the incidence of disease manifestations, including cirrhosis, during subsequent follow up. However, as stated by a recent expert panel, both studies have limited follow-up and mortality data and cannot definitely determine the long-term relationship between iron depletion and outcome [19].

Table 2
Changing patterns of presentation in patients with HHC

| Period | Proportion of patients with: | |
|-----------|------------------------------|------------------|
| | No symptoms (%) | No cirrhosis (%) |
| 1947–1969 | 6 | 20 |
| 1970–1981 | 25 | 48 |
| 1982–1991 | 30 | 58 |

Data taken from Niederau et al. [9].

Other studies have shown a lower percentage of affected individuals with life-threatening complications at diagnosis [9,91]. Niederau et al. [9] found a significant increase in the proportion of patients without symptoms or cirrhosis when diagnosed between 1982 and 1991 compared with those diagnosed between 1947 and 1969 (Table 2). However, it is not clear whether this is an effect of screening or of increased case detection due to greater clinician awareness about HHC.

11. Future prospects

Studies such as those reported by Olynk et al. [32], Bradley et al. [42] and Bulaj et al. [84] which identified new HHC patients with established fibrosis or cirrhosis who are clearly at risk of future liver-related complications provide strong support for screening in Caucasian populations. As discussed previously, the cost-effectiveness of screening for HHC is within acceptable guidelines even if only 20% of patients develop life-threatening complications [71].

However, we believe that national screening programs for HHC are unlikely to develop in most countries in the foreseeable future principally because the exact burden of disease is yet to be clarified and we await the outcome analyses (particularly long-term) of screening studies. In the absence of such programs, we suggest that healthy, preferably young (< 40 years) ambulatory primary care populations are the most appropriate group to be screened (bearing in mind, however, that older patients may also have reversible disease). To this end, it is necessary to increase both the public's and doctor's awareness of HHC [92]. Education as to the magnitude of the problem and the availability of simple

screening tests is required as has been the case for other diseases currently screened for such as hypercholesterolaemia and breast cancer [18,19].

The combination of increased awareness among physicians and the public may ultimately lead to the same goal as population screening, namely the early diagnosis and treatment of the disease [74]. Whether the most appropriate initial test will be TS, TIBC saturation or UIBC still requires further evaluation and further studies are underway. The uncertainty about the clinical expression of the disease is likely to be resolved by the population screening studies that are already in progress world-wide.

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