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Practice Guideline Development Task Force of the College of American Pathologists

Hereditary hemochromatosis[1]

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Abstract

Hereditary hemochromatosis is an autosomal recessive disorder, the gene for which occurs in approximately 10% of Americans, most of whom are unaffected heterozygotes. Approximately 5/1000 white Americans are homozygous and at risk of developing severe and potentially lethal hemochromatosis. The disorder affects numerous organ systems, but the most common symptoms are fatigue, palpitations, joint pains, and impotence; the most common signs are those that relate to hypothalamic, cardiac, hepatic or pancreatic dysfunction, including poor cold tolerance, impotence in males, amenorrhea in females, cardiac arrhythmias, dyspnea, edema, hepatosplenomegaly, spider telangiectases, ascites, deformity, swelling or limitation of motion of joints, weight loss, hyperpigmentation. Characteristic abnormalities of laboratory tests include elevated serum iron concentration, high transferrin saturation, elevated serum ferritin concentration, elevated serum transaminases, hyperglycemia and low values for thyroid-stimulating hormone (TSH) and gonadotropins. Death may be the result of cardiac arrhythmia, congestive heart failure, liver failure or liver cancer. Since many of these complications cannot he reversed once they have developed, early diagnosis and treatment are essential. In view of the high prevalence in the American population (prevalence varies with ethnic background), the low cost of diagnosis and treatment, the efficacy of treatment if begun early, and, on the other hand, high costs and low success rate of late diagnosis

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[1] The Practice Parameter for Hereditary Hemochromatosis was adopted by the CAP House of Delegates on March 31, 1993, and the CAP Board of Governors accepted this parameter as official CAP policy on May 14, 1993.

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and treatment, systematic screening for hemochromatosis is warranted for all persons over the age of 20 years. The initial screening should be by measurement of serum iron concentration and transferrin saturation. The practice guideline provides a diagnostic algorithm for cases in which the serum transferrin saturation is 60% or greater. It also provides guidelines for clinical management.

Keywords: Hemochromatosis; Case finding; Phlebotomy; Transferrin; Ferritin

1. Introduction

It should be understood that adherence to parameters does not guarantee a successful outcome. Rather, these parameters are provided as an educational tool to assist physicians in providing quality care. If equally valid parameters or views advanced by other respected groups are applicable, the physician is, of course, free to follow those authorities. Indeed, the ultimate judgment regarding the propriety of any specific procedure must be made by the physician in light of the individual circumstances presented by a specific patient or specimen.

However, physicians are urged to familiarize themselves with the document. Where a physician chooses to deviate from applicable parameters based on the circumstance of a particular patient or specimen, the physician is well advised to make a contemporaneous written notation of the reason for the procedure followed.

The College recognizes that this document may be used by hospitals and other institutions, managed care organizations, and insurance carriers and other payers. However, this document was not developed for reimbursement or credentialing uses. The College cautions that all these uses involve considerations that are beyond the scope of this document.

2. Method

The task force searched the entire MEDLINE database to identify relevant data as well as other available literature published after 1955. Historical references are included where appropriate. The task force did not purposely exclude any references from consideration. References chosen for inclusion were felt to be most representative of the total body of data on the topic. Since the genetic basis of homozygous hereditary hemochromatosis (HH) was established in the 1970s, the task force is unaware of a significant dissenting viewpoint and encourages the review process to present dissent.

The practice parameter represents the task force's collection of guidelines for HH. Guidelines for iron overload diseases will be addressed in a separate document.

The College of American Pathologists (CAP) conducted reality testing on its Practice Parameter for Hereditary Hemochromatosis. Reality testing is the process by which a practice guideline in draft form is reviewed by CAP members, members of the CAP House of Delegates, and/or members of other medical specialty societies for its usefulness and application to the daily practice of pathology or medicine in general. This parameter was reviewed by more than 200 physicians with special representation by internists, hematologists, gastroenterologists, and family practitioners in addition to pathologists. All comments by reviewers were considered individually by the task force and appropriately addressed in this manuscript.

The College acknowledges the Internal Medicine Center to Advance Research and Education (IMCARE) network of internists who contributed to this parameter by reviewing it in its draft form.

2.1. Definition

Hemochromatosis is a heritable disorder, perhaps the most common autosomal recessive disorder in humans of Northern European origin. There is tight linkage between the hemochromatosis allele and the HLA-A locus [1–15]. This means the hemochromatosis gene must be positioned very close to the HLA-A locus on the short arm of chromosome 6. The abnormal hemochromatosis gene and its product have not been identified yet. The mechanism of prolonged, excessive intestinal iron absorption also remains to be clarified.

2.1.1. Old

An outdated definition of hemochromatosis was the presence of massive body iron overload and iron-related organ injury.

2.1.2. New

The current appropriate definition is the presence of 2 HLA-linked hemochromatosis alleles. The hemochromatosis homozygote may not yet be iron-loaded and may have no symptoms or signs of illness. This must be true for children, adolescents, and most young adults in whom the diagnosis of hemochromatosis is rarely established.

2.1.3. Working definition

The hemochromatosis gene is likely to be cloned and isolated soon and its protein product identified. After that, it will be possible to make a firm diagnosis by quantifying the protein or by DNA sequence analysis. Until then, physicians will have to depend on the presence of an elevated percent saturation of transferrin and serum ferritin concentration in the absence of another explanation for elevated results of blood tests of iron stores (see criteria listed in Table 1).

Table 1
Criteria for the diagnosis of HH

(A) Diagnosis of HH requires observation of elevated TS > 60% on at least 2 occasions in the absence of other known causes of elevated TS
(B) Demonstration of iron overload requires ((1) plus (2), or (1) plus (3))
(1) Elevated SF concentration not explained by other known cause
(2) Increased hepatic iron, if liver biopsy performed ((a) or (b))
(a) Increased stainable hepatocellular iron
(b) Increased hepatic iron concentration and hepatic iron index
(3) Increased mobilizable iron
(a) Phlebotomy therapy advised for all with evidence of iron overload, whether or not liver biopsy has been performed
(b) Each unit of blood withdrawn represents about 200 mg iron
(C) Diagnosis of HH in siblings of known cases
(1) Same as (A) and (B)
(2) HLA usually not needed

2.2. *Other disorders associated with iron loading*

Iron-overload is known to occur in some patients who have a disorder such as idiopathic refractory sideroblastic anemia or sporadic porphyria cutanea tarda (Table 2). The iron loading that occurs in some of these disorders may or may not be contributed to by the presence of one or more hemochromatosis alleles.

Table 2
Other conditions associated with iron overload [26–32]

Sideroblastic anemia
Porphyria cutanea tarda
Transfusional iron overload
β-Thalassemia
X-linked iron loading anemia
Pyridoxine-responsive anemia
Pyruvate kinase deficiency
Hereditary atransferrinemia
Neonatal hemochromatosis
African iron overload
Post portocaval shunt
Chronic hemodialysis
Medicinal iron overload

Other conditions associated with iron loading include atransferrinemia and anemias that are alleviated with multiple transfusions, such as homozygous β-thalassemia and other refractory anemias. Obviously there is usually no need to consider the presence of hemochromatosis alleles in these patients.

There are reports of individuals who ingested iron inappropriately for many years and then were found to have massive body iron overload [16–26].

It is possible that some of the patients who become iron-loaded after years of iron ingestion possess one or more hemochromatosis alleles [27–32].

All patients who have unexplained elevation of blood tests of iron stores without a history of multiple transfusions, for example, are candidates for the diagnosis of hemochromatosis.

It is important to consider the need for liver biopsy and the potential value of iron-depletion therapy in these individuals. Some of the conditions associated with iron loading are listed in Table 2. The present discussion of practice guidelines is concerned with the diagnosis and management of HH.

2.3. Case finding

2.3.1. Purpose

The most important reason for identifying individuals with hemochromatosis is to prevent or minimize iron-related organ injury and premature death.

Failure to identify hemochromatosis early and to undertake a vigorous program of phlebotomy is likely to result in serious morbidity, loss of productivity, very high costs of medical care including organ transplantation, and avoidable premature death. With the rapid aging of the US population, the costs of medical care may be expected to escalate rapidly for patients with hemochromatosis who are not diagnosed and treated early.

Hemochromatosis can be identified by measuring blood tests of iron stores. Serum iron concentration, percent saturation of transferrin, and serum ferritin concentration are widely available. Many hemochromatosis homozygotes have symptoms and signs of illness by age 40 years. Early iron-depletion therapy is known to prolong life. For these reasons, screening, identification, and treatment of subjects with hemochromatosis at an early age should be performed with the goal of preventing the debilitating and life-shortening complications of hemochromatosis.

A large proportion of hemochromatosis homozygotes can be identified during the asymptomatic, early stage of hemochromatosis, prior to the occurrence of symptoms, physical signs, and the laboratory abnormalities from iron-associated organ injury [13,14,33–41].

For every newly diagnosed case of hemochromatosis, approximately 0.8 additional cases will be found among first degree relatives — siblings, parents as well as children — in that family.

2.4. Ascertainment

Some hemochromatosis homozygotes are identified after they seek evaluation because they have symptoms or signs of illness. In others the diagnosis is established only after routine blood tests reveal an elevation of serum iron concentration, percent saturation of transferrin, or serum ferritin concentration.

The ascertainment method correlates with the degree of body iron overload and organ injury. Asymptomatic individuals who are identified by screening studies or during routine testing usually have modestly or moderately increased body iron stores, and usually have no evidence of organ injury [34–37]. Subjects in whom hemochromatosis is identified after they seek medical attention because of symptoms of illness usually have heavy iron overload and organ injury [34,36,37].

2.5. Benefits of early diagnosis

Screening the healthy population by routine measurement of blood tests of iron stores permits identification of patients with early hemochromatosis in the pre-symptomatic stage. When individuals are identified to have hemochromatosis only after symptoms occur, organ injury may be present, and the opportunity to treat early and to prevent organ injury may be lost.

In Table 3, the frequency of many indicators of severity of illness is compared in 97 Utah hemochromatosis probands. Of the 97 probands, 38 were found to have hemochromatosis during population screening studies or during routine blood testing; 59 probands were identified because they were sick [36].

Among the 38 probands ascertained during screening studies, only 10% had symptoms of illness, compared to 100% of the 59 sick homozygotes ($P < 0.0001$). Similarly, a much greater percentage of sick homozygotes had arthropathy ($P < 0.0001$) or very high-grade hepatic parenchymal cell stainable iron ($P < 0.0001$) compared to probands in whom hemochromatosis was identified only because they participated in screening studies [36] (Table 3).

The best explanation for the markedly increased frequency of symptoms and signs of illness in symptomatic homozygotes is that their average age was 49 years compared with an average age of only 29 years in probands who were found to have hemochromatosis during screening studies [36].

Table 3
Comparison of frequency of symptoms, physical findings, and laboratory abnormalities in 97 hemochromatosis probands identified because of illness or during screening studies

	Of 59 sick probands (%)	Of 38 screening probands (%)	*P* value
Symptoms			
Asymptomatic	0	90	<0.0001
Abdominal pain	48	5	<0.0001
Weakness, lethargy	54	5	<0.0001
Palpitations	37	5	<0.001
Impotence (males)	25	3	=0.008
Weight loss	17	5	=0.016
Physical findings			
Skin pigmentation	71	19	<0.0001
Arthropathy	48	10	<0.0001
Hepatomegaly	56	3	<0.0001
Cardiomegaly	14	0	=0.037
Lab abnormalities			
Liver enzyme elevation	68	8	<0.0001
Liver iron stain grade 3-4[a]	98	21	<0.0001
Hepatic cirrhosis	42	0	<0.0001
Diabetes mellitus	27	0	<0.001
Documented hypogonadism	16	0	=0.026

From Edwards CQ et al., unpublished data.
[a]On a scale of 0–4, when grade 0–1 is normal [36].

Table 4
Survival (months) in 111 subjects with hemochromatosis related to phlebotomy therapy

	Subjects	No. survival (months)	Mean duration *P* value
Total no.	111		
Iron depletion therapy	85	63	
No iron depletion	26	18	<0.01

From [42].

Table 5
Survival (months) in 163 subjects with hemochromatosis related to phlebotomy therapy

163 subjects	10-year survival(%)	*P* value
Diabetes mellitus	65	
No diabetes	90	<0.02
Hepatic cirrhosis	72	
No cirrhosis	82	<0.053

From [43].

2.6. *Benefits of early treatment*

2.6.1. *Survival*

Survival in subjects who have hemochromatosis is related to: (1) whether or not iron-depletion therapy is performed; (2) the presence or absence of diabetes mellitus; and (3) the presence or absence of hepatic cirrhosis at the time of diagnosis of hemochromatosis.

In a study of 111 individuals with hemochromatosis [42], 85 underwent phlebotomy therapy and 26 did not undergo treatment for various reasons. The mean survival was much higher in the treatment group compared to survival in the untreated group (Table 4).

In another study of survival among 163 individuals with hemochromatosis [43], survival following iron-depletion therapy was related to the presence or absence of diabetes mellitus or hepatic cirrhosis at the time of diagnosis of hemochromatosis (Table 5). Individuals who underwent iron-depletion therapy prior to the occurrence of hepatic cirrhosis or diabetes mellitus have longevity similar to the age- and sex-matched background population.

Table 6
Decreased survival in 85 subjects with hemochromatosis is determined by hepatic cirrhosis

Individuals	Survival (%)			*P* value
	5-year	10-year	20-year	
All subjects	87	81	71	
No cirrhosis	96	93	93	
Hepatic cirrhosis	72	62	46	<0.004

From [44].

Results of a retrospective study of survival in 85 subjects who had been treated for hemochromatosis appeared in a recent report [44]. Individuals who did not have hepatic cirrhosis experienced survival that was similar to the age- and sex-matched normal population (Table 6). The cumulative survival of the entire group of hemochromatosis patients was 87% after 5 years, 81% after 10 years, and 71% after 20 years. Individuals with hemochromatosis who had hepatic cirrhosis were 5.5 times more likely to die than those with hemochromatosis who did not have cirrhosis. The subjects with cirrhosis had a 5-year survival of 72%, 10-year survival of 62%, and 20-year survival of 46%. In this study, non-cirrhotic hemochromatosis patients who had diabetes had normal survival.

2.6.2. Prevention of complications

Some — not all — complications of hemochromatosis may improve following iron-depletion therapy. Control of diabetes mellitus improves in about one-third; liver enzyme elevation returns to normal in 50–90%; and weakness, right upper quadrant abdominal pain, palpitations, hepatomegaly, and heart failure may improve following phlebotomy therapy [34,42–45].

There are reports of a few impotent men with hemochromatosis who experienced return of sexual function following phlebotomy therapy [33, 34,46,47]. Most men with hypogonadism do not experience complete return of sexual function [42,48]. The best explanation for non-reversible hypogonadotrophic hypogonadism in some heavily iron-loaded individuals must have iron infiltration into and fibrosis of the anterior pituitary, resulting in non-reversible injury of gonadotroph cells.

Arthralgias improve in about one-third of subjects, remain unchanged in about one-third, and worsen in about one-third of subjects following phlebotomy therapy [33,42,49,50]. A few individuals experience onset of diabetes or arthritis during or even after iron-depletion therapy has been completed.

There are reports of a few subjects with hemochromatosis in whom hepatic cirrhosis disappeared following phlebotomy therapy [51–54]. Hepatocellular carcinoma may occur many years after thorough iron depletion. Carcinoma of the liver (hepatocellular carcinoma or cholangiocarcinoma) occurs in 10–29% of individuals with both hemochromatosis and hepatic cirrhosis [42,43,55]. Hepatocellular carcinoma rarely occurs in individuals with hemochromatosis who do not have hepatic cirrhosis [56]. Cirrhosis is a very important risk factor for the development of primary hepatic carcinomas in hemochromatosis.

There are reports of excessive frequency of occurrence of non-hepatic carcinomas in subjects who have hemochromatosis [42,55,57,58]. However, not all investigators agree that there is increased risk for non-hepatic malignancies in hemochromatosis patients [43,59].

Table 7
Summary of estimates of frequency of hemochromatosis from 11 studies in 7 countries

Study type	No. studies[a]	Homozygous Hemochromatosis frequency	
		Mean	Range
Family studies	4	0.005	0.003–0.008
Population studies	4	0.007	0.003–0.013
Autopsy studies	3	0.002	0.001–0.002

Adapted from [13,60].
[a]From [13,35,60,62].

It is important to screen the general population for hemochromatosis — this common disorder — in order to identify and to perform iron-depletion therapy while the hemochromatosis homozygote is in as early a stage of the natural history of hemochromatosis as possible.

2.7. Frequency

2.7.1. Variation of frequency within countries

The mean value of estimates of the frequency of hemochromatosis varies between countries, and within some countries [13,60]. A summary of estimates of the frequency of hemochromatosis in 11 published reports appears in Table 7. An overall average estimate of the frequency is 3–5 per 1000 population [13,35,60,61].

Evidence for variation in estimates of the frequency of hemochromatosis in different geographic areas of a population (Sweden) [62–64] and among different ethnic groups within a country (Canada) [65] appear in Table 8. It seems certain that there is a true difference in the frequency of hemochromatosis among Caucasians, Indians, and Inuit in Canada, for example [65].

Possible reasons for variability in estimates of the frequency of hemochromatosis include: (1) different study types (family studies, population screening, autopsy series); (2) different methods; (3) different diagnostic criteria; and (4) true differences that may exist between population groups and in different geographic areas within a country.

A recent publication provided information about iron stores in sub-Saharan Black Africans [31]. Among 36 iron-loaded index cases, 236 relatives were studied with blood tests of iron stores and HLA typing. The purpose of the study was to test the hypothesis that African iron overload was caused by interaction of a non-HLA-linked genetic factor and dietary

Table 8
Variation of estimates of frequency of hemochromatosis in 3 geographic areas of Sweden and among 3 ethnic groups in Canada

	Sex	No. studied	Study type	Homozygote frequency
Sweden: Area				
Central-Ostersund[a]	M	718	Population	0.005
Southern-Malmo[b]	M	8834	Autopsy	0.001
Eastern-Stockholm[c]	M	11 920	Population	0.001
Western-Goteborg[c]	M	1660	Population	0
Canada: Ethnic group[d]				
Caucasians		1105	Population	0.003
Native American Indians		1407	Population	0
Inuit		310	Population	0

Adapted from [13,60].
[a]From [64].
[b]From [63].
[c]From [65].
[d]From [62].

iron. There were some differences between the findings in the Black African index cases and probands who have HLA-linked hemochromatosis.

The histologic changes in the livers of African Blacks were different from the findings of subjects who have hemochromatosis. Among 25 Black African index cases who underwent liver biopsy, 80% had grade 3 or 4 hepatic parenchymal cell stainable iron (normal grade 0–1 on a scale of 0–4). Ninety-two percent (23) of these 25 individuals had hepatic fibrosis, 40% (10) had hepatic necrosis, and 20% (5) had hepatocellular carcinoma. Also, the liver biopsy iron stains of the Africans revealed abundant iron within reticuloendothelial cells of all index cases, compared to absent or only modest amounts of stainable iron expected within hepatic reticuloendothelial cells of individuals with HLA-linked hemochromatosis. None of the Africans possessed an HLA-A3 or B14 HLA alloantigen, whereas 72% of Caucasians with HLA-linked hemochromatosis possess an HLA-A3 antigen. The hypothesis of tight linkage of an iron loading genetic factor and HLA was rejected among sub-Saharan Africans.

The amount of dietary iron ingested by the Africans was often greater than 80 mg iron per day because they drank home-brewed beer with an iron content of at least 80 mg iron per l. The dietary iron intake among Caucasians in the West is 15–20 mg per day. Mean values of serum ferritin concentration

were higher among first-degree relatives of iron-loaded African index cases than among other family members.

The authors concluded that iron overload among sub-Saharan Blacks was caused by interaction of a non-HLA-linked iron loading genetic factor and the availability of a large amount of iron in home-brewed traditional beer.

More information is needed on the topic of variation in the frequency of hemochromatosis among different populations within countries.

2.7.2. Variation of frequency in different ethnic groups

In the United States, most individuals known to have hemochromatosis are of European ancestry. Similarly, populations with a high frequency of HLA-A3 have a greater population frequency of known hemochromatosis. Hemochromatosis is thought to be uncommon in individuals from Mexico, in North American Indians, in Black Americans, and among Japanese; tile frequency of HLA-A3 is also quite low [66] in each of these ethnic groups (Table 9).

On the basis of the relative frequencies of blood group and HLA markers and of morphologic variants of mitochondria in Caucasian, African, and African-American populations, the probable frequency of HH in African-Americans is 6–12% of the frequency of hemochromatosis in the US Caucasians, or about 3/10 000 to 6/10 000 [67–71]. This estimate implies that HH may not be as rare in African-Americans as is often assumed [72]. Systematic surveys should be performed in African-Americans.

2.7.3. Frequency in US population

The number of individuals with hemochromatosis has been estimated at about 5 per 1000 in the US Caucasian population. If there are 200 000 000

Table 9
Frequency of HLA-A3 among various populations

Racial and ethnic background	%
European Caucasians	26
Australian Caucasians	24
African Blacks	12
North American Blacks	18
Ashkenazic Jews	16
Mexicans	6
North American Indians	3
Japanese	1

Adapted from [66].

US Caucasians, this means that about 1 000 000 individuals in the US alone should be hemochromatosis homozygotes, and are at risk to develop iron-related organ injury.

Only a small literature exists concerning iron overload among African-Americans. A recent report described the occurrence of iron overload in a Black American who had sickle cell disease [28]. Iron overload was not due to transfusion; the patient had received only 2 units of blood transfusion.

The patient was a 51-year-old man who had arthralgias, glucose intolerance, markedly elevated values of transferrin saturation (96%) and serum ferritin concentration (2450 ng/ml), heavy hepatic iron overload, and hepatic cirrhosis. His increased amounts of stainable hepatic iron was predominantly within hepatocytes, rather than in reticuloendothelial cells. He possessed an HLA-A3 alloantigen.

The author concluded that this Black American was homozygous for sickle disease and also was homozygous for HLA-linked hemochromatosis.

A recent abstract provided results of a retrospective autopsy study of hepatic iron stores among US Blacks who underwent autopsy [73]. The majority of the stainable hepatic iron was present within reticuloendothelial cells, rather than within hepatocytes. These results are more like the distribution of hepatic iron among sub-Saharan Africans than among US Caucasians who have HLA-linked hemochromatosis.

A report recently submitted for publication included results of assessment of serologic and hepatic tests of iron stores among 10 African-Americans [74]. Overall, the African-Americans had lower mean values of transferrin saturation, greater amounts of stainable iron with hepatic reticuloendothelial cells, and absence of an HLA-A3 antigen, compared to US Caucasians with hemochromatosis.

The authors concluded that iron loading among US Blacks is more like the iron loading described among sub-Saharan Blacks than the iron loading in Caucasians who have HLA-linked hemochromatosis.

There is a paucity of information about iron overload among African-Americans. More study will be required before this disorder can be described adequately.

2.7.4. Variation in frequency between sexes

There is a major difference in the frequency of the diagnosis of hemochromatosis between the sexes in all countries (Table 10). No known study includes an equal number of males and females [1,3,37,43,75–82]. This presumably is due to the smaller amounts of body iron stores in women.

The amount of iron that can be mobilized during phlebotomy therapy is about 2.5 times greater in a group of males compared to a group of females

Table 10
Male to female ratio of subjects with hemochromatosis (HC) in 14 studies

Author	Year	Study type	HC total No.	Males No.	Ratio M:F
Bomford, Williams [42]	1976	All affected	111	110	110:1
Sheldon [75]	1935	Lit. survey	311	295	18:1
Panajotopoulos [76]	1989	Probands	67	62	12:1
Finch, Finch [77]	1955	Lit. survey	787	711	9:1
Wiggers et al. [80]	1991	Probands 1	0	9	9:1
Niederau et al. [43]	1985	All affected	163	145	8:1
Porto et al. [78]	1989	Probands	17	15	7:1
Simon et al. [3]	1977	Probands	97	85	7:1
Milman [81]	1991	All affected	179	140	4:1
Saddi, Feingold [1]	1974	Probands	96		4:1
Powell et al. [79]	1990	All affected	91	61	2:1
Adams et al. [37]	1991	All affected	93	60	2:1
Czink et al. [82]	1991	Probands	13	8	1.6:1
Edwards et al.[a]	1992	all affected	205	127	1.6:1

Adapted from [13].
[a]Unpublished results.

with hemochromatosis [33,34]. This probably is related to physiologic sources of iron loss in women (menses, pregnancies).

However, some females with hemochromatosis have greater body iron stores than some men with the disorder, so women cannot be ignored in hemochromatosis screening programs. Some women with hemochromatosis develop organ injury and die from chronically neglected iron overload.

2.7.5. Age at which to screen for hemochromatosis

The optimal age at which to test an individual or a population for hemochromatosis has not been established. It is known that some children have been found to have hemochromatosis, indicating that some homozygotes have elevated blood tests of iron stores as young as age 2 years [38–41,83,84]. It seems reasonable to screen young adults for hemochromatosis, perhaps at age 20 years. In addition, children of known cases and siblings of known cases should be tested every 5 years. It is anticipated that this screening strategy will result in identification and treatment of a large number of asymptomatic homozygotes.

Table 11 indicates a sequence that may be followed as a screening strategy for hemochro matosis [85].

Table 11
Stepwise strategy for screening and treatment of hemochromatosis

TS > 60% ↓ SF		
↓ Abnormal for age and sex		↓ Normal
↓ Liver biopsy accepted	↓ Liver biopsy refused	↓ Repeat every 2 years Initiate phlebotomy if ferritin > normal
↓ Phlebotomy	↓ Phlebotomy	or
Initial: rapid sequence Lifelong: maintenance Measure ferritin every year and adjust frequency of phlebotomy to maintain normal ferritin		Initiate phlebotomy 3-4 units/year Measure ferritin every 2 years and adjust frequency of phlebotomy to maintain normal ferritin

Adapted from [85] (in revision).

2.8. Subjects in whom the diagnosis should be considered

2.8.1. Asymptomatic individuals

2.8.1.1. Elevated blood tests of iron stores. Individuals who are found during routine serum chemistry screening tests to have an elevation of percent saturation of transferrin or serum ferritin concentration are good candidates for the diagnosis of hemochromatosis, if they do not have an obvious explanation for iron overload, such as blood transfusions. Repeat measurement of serum iron concentration, percent saturation of transferrin, and serum ferritin concentration is important in these individuals.

Screening for hemochromatosis using percent saturation of transferrin and serum ferritin concentration is likely to identify 2 groups of hemochromatosis homozygotes (Table 11): those with elevation of both transferrin saturation and serum ferritin concentration, and those with elevation of transferrin saturation alone [35,86]. Liver biopsy is advisable in patients with elevation of both transferrin saturation and serum ferritin concentration. The purpose

of liver biopsy is to determine the amount of hepatic iron overload, and to assess hepatic injury and/or cirrhosis. The next option among patients who refuse liver biopsy (Table 11) is to start a life-long course of phlebotomy therapy to eliminate excessive iron stores in an attempt to decrease the high risk of developing organ injury [85].

Individuals who have elevated transferrin saturation with normal serum ferritin concentration may be followed with measurement of serum ferritin concentration at 1- or 2-year intervals. The purpose of this is to determine when body iron stores start to increase, so further evaluation can be performed, and so life-long, intermittent phlebotomy therapy can be initiated before organ injury occurs [85].

Those who have persistent elevation of transferrin saturation (greater than 60%) and elevation of serum ferritin concentration (greater than 400 ng/ml in men; greater than 200 ng/ml in women) should be considered for liver biopsy to determine the amount of stainable iron and to assess for injury. Individuals who have higher values of serum ferritin concentration are likely to have greater amounts of hepatic parenchymal cell stainable iron and hepatic iron concentration. It has been demonstrated that each 1 μg/l (1 ng/ml) of serum ferritin concentration represents 7.5 mg of mobilizable body iron [87–91]. This relationship should not be expected to hold true in individuals whose ferritinemia is caused by an inflammatory process that increases serum ferritin concentration beyond the effect of hepatic iron overload alone [33].

Healthy, asymptomatic individuals who have elevated results of blood tests of iron stores should be followed up with further testing for hemochromatosis unless there is a clear alternative explanation for the elevated results. Elevated results of blood tests of iron stores should not be ignored or dismissed as though they are not important.

2.8.1.2. Elevated concentrations of liver enzymes in serum. Individuals who have elevated serum concentrations of liver enzymes but do not have a history of alcohol abuse, proven recent hepatitis, or use of medications known to cause elevations of liver enzymes, are candidates for the diagnosis of hemochromatosis. Measurement of serum iron concentration, percent saturation of transferrin, and serum ferritin concentration is important in these people.

A recent study was designed to determine the cause of elevated concentrations of liver enzymes in serum in 149 apparently healthy subjects [92]. In each case, liver enzymes were measured routinely for insurance, work, or reasons other than symptoms of illness (aspartate aminotransferase and alanine aminotransferase). Results of liver biopsy demonstrated that 3.4% of these subjects (5 of 149) had hemochromatosis [92]. This high figure indicates the importance of further evaluation of individuals who have unex-

plained elevation of liver enzymes in serum. Serum ferritin concentration was greater than 300 μg/l in these 5 patients, but not in the other 144 subjects.

A recent report demonstrated that 65 out of 100 subjects with hemochromatosis had elevation of aspartate aminotransferase (AST, SGOT) and/or alanine aminotransferase (ALT, SGPT) [93]. The elevation usually was modest, only 1–2 times greater than normal in non-cirrhotic subjects, and up to 6 times the upper limit of normal in cirrhotic patients with hemochromatosis.

2.8.1.3. Blood donors. Healthy subjects who voluntarily donate blood and who have unexplained elevations of liver enzymes in serum are candidates for the diagnosis of hemochromatosis [35]. Similarly, individuals who are able to maintain a normal hematocrit even after donating many units of blood for transfusion also may be good candidates for the diagnosis of hemochromatosis. Measurement of serum iron concentration, percent saturation of transferrin, and serum ferritin concentration is important in these people.

2.8.1.4. Increased amounts of stainable iron in liver biopsy specimens. There are many reasons why individuals undergo liver biopsy. All liver biopsy specimens should be stained for iron. Some individuals who undergo liver biopsy are found to have increased amounts of stainable hepatocellular iron, even when the preoperative diagnosis may not have included hemochromatosis. These individuals should be considered to be candidates for hemochromatosis.

Normal individuals have grade 0–1 hepatic parenchymal cell stainable iron. Subjects who have hemochromatosis usually have grade 3–4 stainable liver iron, though some young homozygotes have normal or near-normal stainable liver iron [35].

The finding of markedly increased amounts of hepatic parenchymal cell stainable iron should be considered an important finding that should lead to treatment of hemochromatosis. The finding of an increased amount of stainable liver iron should not be ignored or dismissed as unimportant.

2.8.1.5. Hepatic iron concentration. Determination of hepatic iron concentration has value in estimating the amount of body iron overload. This measurement can be performed by atomic absorption spectrophotometry. Normal individuals have 100–2200 μg iron/g liver dry weight. Normal values of hepatic iron concentration from 6 studies appear in Table 12 [94–99].

2.8.1.6. Hepatic iron index. There is increasing information about the value of calculating the hepatic iron index in individuals who undergo liver biopsy, in an attempt to interpret the significance of liver iron content adjusted to the age of the patient [98,100–103]. The hepatic iron index is measured by dividing hepatic iron concentration (in μmol iron/g dry weight) by the individual's age in years. Normal subjects have a hepatic iron index of 1.1 or less;

Table 12
Hepatic iron concentration values in normal individuals in 6 studies

Study	Hepatic iron concentration (μg iron/g dry weight)				Males normal values	Females normal values
	Males		Females			
	No.	Normal values	No.	Normal values		
Barry and Sherlock [94] 1971	10	<1364	7	<1304	<25[a]	<24[a]
Brissot et al. [95] 1981		<2000[b]		<36[b]		
Chapman et al. [96] 1982	16[b]	1400[a,b]				<25[a,b]
LeSage et al. [97] 1983	15	724 + 278[d]	6	724 + 278[d]	<23[a]	<23[a]
Bassett et al. [98] 1986	40[b]	<1840			<33[b]	
Moyer et al. [99]	40	300–2200	40	100–1800	<40	<33

[a]Systeme Internationale (SI) units not presented in original; SI values (μmol iron/g liver dry weight) were calculated from results of μg iron/100 mg (or per g) liver dry weight.

[b]Results not presented for each sex; # of males, # of females not stated.

[c]Mean value ± S.E.M. presented, not upper limit of normal.

[d]Mean value ± S.D. presented; mean + 2 S.D. = 1280 μg iron/g dry weight.

[e]Moyer TP, Baldus WP, Fairbanks VF et al. Unpublished. In this follow-up study of 80 adult victims of accidental death, 38 males had hepatic iron concentration of 200–2200 μg/g dry weight. The median value for males was 1030 μg/g. Two other male cases were high outliers with values of 2400 and 2600 μg/g. Thirty-nine adult women had values ranging from 94–1600 μg/g. The median value for women was 633 μg/g. In this group of women, there was one high outlier with a value of 2400 μg/g.

those with alcoholic liver disease, 1.7 or less; hemochromatosis heterozygotes, 1.9 or less; and hemochromatosis homozygotes, 1.9 or greater.

An example of the 2 steps involved in the calculation of hepatic iron index appears in Table 13.

Data from 5 studies in the literature with information about the possible usefulness of the hepatic iron index appear in Table 14.

2.8.1.7. Other methods of estimating body iron stores. Computed tomography, magnetic susceptometry, magnetic resonance imaging, and deferoxamine-induced urinary iron excretion are additional methods that can be

Table 13
Two-step method of calculation of HII in a hypothetical patient

What is the hepatic iron index value in a 30-year-old whose hepatic iron concentration is 5022 μg iron/g dry weight liver?			
Step (1)	Convert μg iron to μmol iron:	1 μmol iron / 55.8 μg/μmol 5022 μg	= 55.8 μg iron = 90 μmol iron
Step (2)	Divide μmol iron by age:	90 / 30	= 3.0
The hepatic iron index in this individual is 3.0, which is greater than the upper limit of normal, compatible with hemochromatosis.			

employed to estimate body iron stores [102,104–113]. These tests are either too insensitive, too costly, or are not widely available. Therefore, none of these are appropriate tests to use for screening the general population. These test methods will not be discussed in detail here. References are included for interested readers [102,104–113].

2.8.2. Sick individuals

2.8.2.1. Candidates for the diagnosis of hemochromatosis. Individuals who have hemochromatosis often have symptoms and signs of illness. The results that appear in Table 15 indicate the frequency of symptoms, physical examination findings, and laboratory abnormalities in hemochromatosis patients from 6 studies in the literature [34,58,75,77].

Table 14
HII in normal individuals, subjects with alcoholic liver disease (ALD), hemochromatosis heterozygotes (Hh), and in hemochromatosis homozygotes (hh)

Study	Normal	ALD	Hh	hh
Bassett et al. [98]	<1.1	<1.5	<1.9	>2.3
Summers et al. [100]			<1.5	>1.9
Olynyk et al. [101]		<1.7		>2.0
Bonkovsky et al. [102]	<0.8	<1.2	<1.9	>2.3
Sallie et al. [103]	<1.7			>2.0

Hepatic iron index is calculated by dividing the hepatic iron concentration (in μmol iron/g liver dry weight) by the age of the patient (in years).

Table 15
Frequency (%) of symptoms, physical findings and laboratory abnormalities in patients with hemochromatosis from 6 studies

Type of study	Sheldon [75] Lit. review	Finch [77] Lit. review	Milder [58] Index cases	Utah [34] Family studies	Niederau [43] All patients	Adams [37] Family studies
Number of patients	311	787	34	41	163	93
Males	295	711	34	26	145	60
Females	16	76	0	15	18	33
Symptoms (% of patients)						
Asymptomatic			15	37		24[f]
Weakness or fatigue	13	70	73	22	83	32
Weight loss		44	53	7		
Arthralgias			47	56	43	45
Abdominal pain	26	29	50	20	58	10
Loss of libido/impotence	6	14	56	24[b]	38	
Cardiac complaints			33	35	39	
Physical and lab findings (% of patients)						
Skin pigmentation	84	85	82	49	75	27
Hepatomegaly	92	93	76	54	83	38
Abnormal liver enzymes			54	61	43	
Cirrhosis	92		94	41	69	30
Hepatocellular carcinoma	6	14	18	2.4	10	1
Splenomegaly	55	50	38	37	13	
Diabetes	79	82	53	12	55	18
Testicular atrophy		16	50	20		
Hypogonadism, documented			40[c]	20[d]		24[g]
Hypogonadotrophic hypogonadism		100[c]	80[d]			
Arthropathy			44	68		
Hypothyroidism				8[e]		
Cardiac arrhythmia		35	26	7	36	
Congestive heart failure		33	35	2.4	12	5

[a]Adapted from [34].
[b]Of 26 men, 6 had testicular atrophy.
[c]Of 10 men tested, 4 had hypogonadism — all of the secondary type.
[d]Of 26 men tested, 5 had documented hypogonadism; 1 of these 5 had primary and 4 had secondary hypogonadism.
[e]Of 34 men tested, 3 had hypothyroidism. Of 15 women tested, none had thyroid disease. Total number tested = 49 [114].
[f]Percentages in this study calculated from data presented in text.
[g]Of 60 men.

Table 16
Clinical findings, complications, specialty of physicians, or clinical practice setting likely to include hemochromatosis patients, in some of whom the correct diagnosis may not yet be established

Clinical problem	Specialty
Amenorrhea	Endocrinology
Diabetes mellitus	Family practice
Hypopituitarism, hypogonadism	Gynecology
Impotence, loss of libido (males)	Internal medicine
Infertility (males, females)	Obstetrics
Premature menopause	Psychiatry
Thyroid disease: hypothyroidism	Urology
Ascites	Family practice
Esophageal varices	Gastroenterology
Hepatic cirrhosis	General surgery
Hepatic failure	Internal medicine
Hepatocellular carcinoma	
Hepatomegaly	
Liver disease, unexplained elevation of liver enzymes, especially AST (SGOT) and ALT (SGPT)	
Portal hypertension	
Koilonychia	Dermatology
Porphyria cutanea tarda	Family practice
Skin pigmentation: grey or bronze	Gastroenterology
Telangiectases	Internal medicine
Arrhythmias: supraventricular, ventricular	Cardiology
Arthropathy: 2nd or 3rd	Family practice
Metacarpophalangeal joints	Internal medicine
Knees: with or without chondrocalcinosis	Orthopedics
Hips	Rheumatology
Cardiomegaly, cardiomyopathy	Thoracic surgery
Hypertrophic or dilated type	
Congestive heart failure	
Pseudogout	
Seronegative rheumatoid arthritis	
Unusual color of excised cartilage or synovium	
β-Thalassemia minor	Hematology
Iron-loading anemias	Internal medicine
Sideroblastic anemia	Pathology
Hepatic cirrhosis without hepatitis	Pathology
Hepatocellular carcinoma, specimen not stained for iron or synovium with brown or grey hue	
Previously obtained liver specimen not stained for iron	

Table 16 (*Continued*)

Susceptibility to systemic infection [115-116]	Infectious disease
Alcoholics with increased liver iron	All specialties
Elevated percent saturation of transferrin or SF	

Clinical problems in these clusters may be encountered by specialists in clusters on right side of table.

The data in Tables 15 and 16 [114–116] emphasize the possibility of hemochromatosis as a cause of many complaints or abnormal findings in a patient. Subjects who have any of these findings are candidates for the diagnosis of hemochromatosis unless another cause has been proved. Measurement of serum iron concentration, percent saturation of transferrin, and serum ferritin concentration is important in these people.

2.8.2.2. Clinical setting for the diagnosis of hemochromatosis. The symptoms for which any undiagnosed subject with hemochromatosis will seek medical attention cannot be predicted in advance, obviously. For this reason, an affected individual with the disorder may be seen and evaluated by physicians in many specialties (Table 16). To establish a correct diagnosis, it is necessary for the physician to consider the possibility of hemochromatosis in a patient with any of the symptoms or signs of illness included in Tables 15 and 16. It is also necessary that physicians obtain confirmatory tests in all individuals who are found to have elevations of percent saturation of transferrin or serum ferritin concentration.

Table 17
The frequency of HLA-A3 in the general population is so high that any individual who possesses the HLA-A3 alloantigen is much more likely to be a normal homozygote than a hemochromatosis homozygote

	Frequency of + HLA-A3		Population frequency
Non-hemochromatosis	28%	= 280/1000	
Hemochromatosis	71%		0.5% (5/1000)
	(0.71 × 5/1000)	= 3/1000	
	280 – 3/1,000	= 277/1000	

Note: Of 280 subjects/1000 in the general US Caucasian population who possess an HLA-A3 alloantigen, 277/280 (99%) do not have hemochromatosis. HLA typing is not a useful test for screening the general population for hemochromatosis.

2.9. Evaluation of relatives

2.9.1. Pedigree studies

The highest concentration of new cases of hemochromatosis will be found among the siblings of individuals who have the disorder: within a family every sibling has a 1-in-4 chance of having hemochromatosis. The frequency of heterozygosity for hemochromatosis in the general population is estimated at about 13%, based on an estimate of the frequency of homozygosity for hemochromatosis of 5 per 1000 in the general US Caucasian population (Table 17). This means that 1 out of 8 people with hemochromatosis will marry a heterozygote. Since 50% of the offspring of homozygous-heterozygous matings will be hemochromatosis homozygotes, the high population frequency of homozygosity and heterozygosity account for the somewhat frequent observation of hemochromatosis in consecutive generations, which mimics dominant inheritance.

Homozygous-heterozygous matings are understandably common. If a parent of a hemochromatosis patient also has hemochromatosis, 50%, rather than 25%, of the patient's brothers and sisters will have the disorder. Some children have been found to have hemochromatosis, indicating that some homozygotes have elevated blood tests of iron stores as young as age 2 years [38–41,83].

For the reasons stated here, it is important to screen for hemochromatosis among all the first-degree relatives of individuals who have the disorder, including siblings, parents, offspring, and also spouses.

The finding of an elevated transferrin saturation greater than 60% and a serum ferritin concentration greater than 400 ng/ml in men or greater than 200 ng/ml in women should be considered as evidence of hemochromatosis unless another known cause of elevated blood tests of iron stores is present. Elevated results of these tests should suggest the need for further evaluation and consideration of the advisability of liver biopsy.

2.9.2. Tests of iron stores

The screening tests to use in evaluation of family members are the same as for screening the general population — percent saturation of transferrin and serum ferritin concentration.

It should not be expensive to add measurement of serum iron concentration and the total iron binding capacity to the automated, multiple serum chemistry analysis that is commonly run at the time of intermittent health screening. The laboratory should be able to measure serum ferritin concentration automatically on serum samples that have a transferrin saturation greater than 50% (or lower than 15%). If this sequence of testing becomes the established method for screening the population, practicing physicians will

have important and usable information about their patients' iron status. It is anticipated that this will lead to the diagnosis of hemochromatosis in many previously unsuspected individuals — hopefully before organ injury occurs [117].

Relatives who have elevated results of blood tests of iron stores should be advised to undergo liver biopsy to quantify iron stores and to assess hepatic integrity or injury histologically.

2.10. HLA typing

HLA typing is not a useful screening test for hemochromatosis in the general population for the following reasons [118]: (1) the specific HLA antigens and haplotypes associated with hemochromatosis vary between families; (2) HLA typing is very expensive compared to blood tests of iron stores; (3) HLA typing does not provide any information about iron stores in the person tested; and (4) most individuals in the general population who possess the HLA-A3 alloantigen, for example, do not have hemochromatosis.

The frequency of HLA-A3 in the general Caucasian population is known to be about 28%, or 280 per 1000. About 71% of individuals with hemochromatosis possess an HLA-A3 antigen [97,119]. Given an average population frequency of hemochromatosis of 4 per 1000, 3 of the 4 individuals per 1000 who have hemochromatosis will possess an HLA-A3 antigen (Table 18).

This means that of the 280 individuals per 1000 population who possess an HLA-A3 antigen, only 3 per 280 of them will have hemochromatosis and the remaining 277 individuals per 1000 do not have hemochromatosis. The probability is 99:1 that HLA testing of the general population will identify

Table 18
Comparison of hepatic iron concentration (mean values) in alcoholics, subjects with hemochromatosis, and normal individuals

Subjects	No.	Hepatic iron concentration	
		(μg iron/g dry weight)	(μmol iron/g dry weight)[a]
Alcoholics	60	<1594	<28
Hemochromatosis	15	20945	375
Normal		<1400	<25

From [96].

[a]Systeme Internationale (SI) units not presented in original; SI values (μmol iron/g dry weight) were calculated from results of μg iron/100 mg dry weight.

Table 19
Comparison of frequency of HLA-A3 in alcoholics, subjects with hemochromatosis, and normal individuals

Subjects	Number tests		% with HLA-A3	
	France[a]	USA[b]	France[a]	USA[b]
Alcoholics	22	50	29	28
Hemochromatosis	38	56	76	70
Normal	204	1208	23	28

[a]From [119].
[b]From [97].

normal people who possess an HLA-A3 antigen but do not have hemochromatosis (Table 17).

HLA typing may be useful in evaluating very young siblings of subjects who have hemochromatosis. It is only appropriate to perform HLA typing if the young siblings have normal percent saturation of transferrin and serum ferritin concentration. HLA-identical siblings of affected individuals are also considered to be homozygous for hemochromatosis. These relatives should be followed with intermittent blood tests of iron stores in order to initiate iron-depletion therapy when serum ferritin concentration becomes elevated. Again, periodic determination of transferrin saturation and serum ferritin

Table 20
Alcoholics who have grade 3-4 hepatic parenchymal cell stainable iron also have markedly elevated values of hepatic iron concentration and increased frequency of HLA-A3, indistinguishable from hemochromatosis

Subjects	No.	Mean values of hepatic iron concentration		% with HLA-A3
		(μg iron/g dry weight)	(μmol iron/g dry weight)	
Iron overload				
with alcohol abuse	20	17 420	312	74
without alcohol abuse	41	28 530	511	68
Normal males		724	13	28
Cirrhosis	50			28

From [97].

concentration can accomplish the same thing without the great expense of HLA typing. This is a very limited indication for performance of HLA typing among relatives of individuals who have hemochromatosis.

2.11. Evaluation of alcoholics

As a group, alcoholics who undergo liver biopsy usually have normal or only slightly increased amounts of hepatic parenchymal cell stainable iron (grades 0–2) compared to normal individuals and subjects who have hemochromatosis [76,94–97,119–120] (see Tables 18–20). Alcoholics who have markedly elevated amounts of stainable liver iron (grades 3–4) have HH in addition to alcoholism — not just increased storage iron due to alcohol abuse.

If hepatic iron concentration has been measured, a value 5 or 6 times the upper limit of normal is characteristic of subjects with hemochromatosis. Individuals who have alcoholic liver disease without hemochromatosis usually have near-normal hepatic iron concentration (Tables 18–20). Normal values for hepatic iron concentration appear in Table 12.

Iron overload in these patients should not be ignored; rather, these individuals should undergo iron depletion therapy. In addition, their first-degree relatives should undergo measurement of serum iron concentration, percent saturation of transferrin, and serum ferritin concentration. Relatives who have elevated results of blood tests of iron stores need further evaluation for hemochromatosis.

2.12. Evaluation of patients with chronic hepatitis

Some individuals with chronic hepatitis have elevated results of serum and hepatic tests of iron stores. Possible explanations for the elevated test results might include hepatic necrosis alone, or the occurrence of viral hepatitis in an individual who has hemochromatosis [121–124].

Results of serum and hepatic iron measurements in 28 subjects with chronic hepatitis were reported recently [125]. Thirteen (46%) had elevated serum iron concentration; 13 (46%) had elevated percent saturation of transferrin; 12 (43%) had elevated serum ferritin concentration; only 4 (14%) had increased stainable hepatic iron; only 4 (14%) had elevated hepatic iron concentration. Only 2 individuals (7%) had a hepatic iron index greater than 2.0. It is possible that calculation of the hepatic iron index can be employed among patients with chronic hepatitis to help identify those who have coincidental hemochromatosis.

Measurement of transferrin saturation and serum ferritin concentration among first-degree relatives of patients with high-grade hepatic stainable

iron and/or hepatic iron concentration, and/or hepatic iron index may result in the detection of unsuspected relatives who have hemochromatosis. Identification of a relative with hemochromatosis may corroborate the diagnosis of HH in the index case.

2.13. Hemochromatosis heterozygotes

Most heterozygotes have normal blood tests of iron stores, but some have elevated results [86,87,126]. Sometimes it is difficult to determine if an individual with elevated test results is a homozygote who has less iron than most heterozygotes [86,87,126]. It is possible that variable expressivity influences the iron stores in some heterozygotes and some homozygotes [127]. Iron ingestion in medicines may increase the amount of body storage iron enough to cause elevated test results of iron stores [19–25]. In the event that an individual has elevated results of tests of iron stores intermediate between values usually found in homozygotes and heterozygotes, it seems reasonable to consider phlebotomy therapy to prevent the future possibility of iron-induced organ injury. This may result in a short course of phlebotomy therapy of some heterozygotes who would not go on to accumulate a heavy body iron burden, but it seems unsafe to ignore the possibility that such patients may be homozygous and at risk for the serious morbidity of HH.

3. Performance characteristics of laboratory tests

Fortunately, the most widely available tests of iron metabolism are also the most predictive of homozygous HH. Although measurement of serum iron (SI) concentration alone is an insensitive indicator of homozygous HH, when divided by a measure of serum transferrin concentration to yield percent transferrin saturation (TS), both sensitivity and specificity for detection of homozygous HH are improved [126,128–130]. Serum ferritin, the other widely available serum test, is less predictive than TS, [126] but may improve predictive value when interpreted in conjunction with TS [128,129].

TS can be derived by measuring transferrin by any of 3 methods: (1) total iron binding capacity (TIBC); (2) unsaturated iron binding capacity (UIBC) [130,131]; (3) immunochemical measurement.

TIBC is labor-intensive owing to separation techniques ($MgCO_3$ or resin) for removing the excess iron added to fill transferrin binding sites in the first step of the procedure. This separation is required when iron is measured at low pH. UIBC is easily and inexpensively totally automated owing to the measurement of the added iron in excess of that required to saturate transferrin at a reaction pH compatible with iron binding by transferrin. This technology is discussed elsewhere [130,132,133]. Immunochemical transferrin

measurement correlates well with TIBC, is less frequently used, and has not been extensively studied in homozygous HH [134,135].

The following equation shows the calculation of TS:

$$TS\ (\%) = SI/SI + UIBC$$

or

$$TS\ (\%) = SI/TIBC$$

Serum tests of iron metabolism are subject to preanalytical, analytical, and within-individual biologic variability. Ingested iron will increase postprandial SI concentration. The increase is larger in iron-deficient individuals than in those who are iron-replete as reflected in the iron tolerance test [136]. A 20-mg oral dose causes an increase in SI concentration of 110 μg/dl (19.7 μmol/l) in iron-deficient subjects and 28 μ/dl (5.0 μmol/l) in normals. Therefore, interpretation of SI must consider recent food or therapeutic iron ingestion. Short-term variations in serum ferritin and TIBC have not been reported with meals or therapeutic iron.

Imprecision in SI measurements exists within every laboratory and there are differences between laboratories. For SI, a typical between run intralaboratory coefficient of variation (CV) is 4–6% [137]. However, 5% of laboratories have CVs greater than 10%. Between laboratories using similar methods, a 9–15% CV is to be expected (CAP Survey 1990–1991) [138]. The intralaboratory and between laboratory analytical imprecision for TIBC is inherently greater than for SI owing to the additional manual pipetting and separation steps.

There are sytematic differences between iron methods. Results from the 1991 CAP C-A survey indicate these are less than 10% for all methods subscribing except the Kodak Ektachem and Technicon SMAC methods. The mean value for Kodak Ektachem users was 19–35% lower than the all method mean observed by 1463 participants using similar chromophores. These differences have been attributed to substances in the survey material, which produce matrix interference. The Kodak method bias disappears when fresh patient samples are analyzed [139]. Method-to-method differences for SI, TIBC, and ferritin require method-specific reference ranges.

Clinical laboratory methods for SI were studied extensively using 1977-1978 CAP survey data [140,141]. Gilbert [140] compared the CAP all participant mean to that of the results of the National Bureau of Standards (NBS) isotope dilution mass spectrometry assay, which has a CV of 2%. For 9 control samples with NBS determined values between 56.6 μg/dl (10.1 μmol/l) and 92.1 μg/dl (16.5 μmol/l), the differences ranged from 0 (0%) to −3.7 μg/dl

(0.66 μmol/l or 4%). For 3 control samples with NBS values between 150.3 μg/dl (26.9 μmol/l) and 158.5 μg/dl (28.4 μmol/l), the differences were −18.6 μg/dl (3.3 μmol/l or 12.4%) to −21.2 μg/dl (38 μmol/dl or 13.4%). A similar relationship was observed for each individual method. Gilbert concluded that the methods were similar and choice of color reagent was unimportant. The most accurate method was the Technicon Auto Analyzer, which used dialysis to eliminate interferences due to serum proteins. Itano [141] agreed the color reagent was not important but interference by protein probably contributed to inaccuracy. No later comparison of clinical iron methods to definitive methods has been found.

Gilbert's [140] data show that in 1978 most laboratories underestimated SI by 12% at 150 μg/dl (26.9 μmol/l). In hemochromatosis, there is an equivalent deviation from the true value both for SI and for TIBC. Since the underestimation is proportional for both SI and TIBC, the TS result should not be affected. Of course, this will not be perfectly true, but the methods in current use are judged acceptable for identifying individuals requiring additional investigation for hemochromatosis.

In 1990 the National Committee for Clinical Laboratory Standards (NCCLS) published H-17P, the proposed standard for determination of SI and TIBC [142]. The manual NCCLS method precipates protein prior to spectrophotometric measurement of iron using Ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid) 1,2,4-triazine]. This guideline will not specify iron measurement methods. The committee is cognizant of the need for continuing quality improvement among these methods [143,144].

Analytical interferences with iron measurements have been tabulated [145]. Each method has specific limitations. Interferences from sample hemolysis should be well understood in any method used. Each laboratory must examine its method and determine the degree of interference caused by storage, freezing, and/or hemolysis. The NCCLS [142] method indicates that more than minimally hemolyzed samples are unacceptable.

Between-day and within-day variability of SI, TIBC, and ferritin in normal volunteers has been studied. The intraindividual CV for SI, TIBC, and serum ferritin were 28.5, 4.8, and 14.5% in 13 healthy volunteers studied daily using morning samples during a 3-week interval [146]. Other reports confirm the wide intraperson day-to-day variation in SI and lesser variation in TIBC and ferritin [147–153]. Much larger variability has been observed in samples collected within a 24-h period [153]. However, published data disagree regarding the pattern of changes between 08:00 and 17:30 [152]. The data of Costongs et al. on 62 volunteers did not show a systematic pattern among values obtained within a single day [152]. Serum ferritin does not show within-day variation [152,153]. However, serum ferritin, like hepatic iron, must be interpreted with respect to patient age [35,154]. Recent data [155]

suggest the current automated method for serum ferritin shows a 97.5th perentile of 215 μg/l for females under 50, 417 μg/l for females over 50, and 408 μg/l for 260 males.

The within-day variability of SI, TIBC, and TS has also been studied in hemochromatosis patients [60]. Patients (n = 43) and normal controls were sampled at 2-h intervals for one 24-h period. SI and TS results were constantly abnormal in the hemochromatosis patients, with little variation between the mean values observed at each sample time. These data support the conclusion that sample timing is unimportant for individuals with homozygous HH. Edwards also studied 3 male and 4 female controls. The serum concentrations were maximal at 08:00–10:00 and minimal at 20:00–02:00 (see Table 21).

The preanalytical variability in SI concentration in response to meals is large enough to alter patient care decisions as discussed below [35]. If there were no preanalytical variability, the analytical variability is sufficiently small to allow accurate diagnosis. Errors attributed to differences between iron methods are somewhat diminished by using the ratio (TS = 100 Fe/TIBC). The intrapersonal day-to-day variability has been shown to be

Table 21
Twenty-four hour variations in SI concentration and TS in normal men and in normal women [27]

		Time of day or night (24-h clock)												
	No.	06	08	10	12	14	16	18	20	22	24	02	04	06
Normal males	4													
SI (μg/dl)														
Mean		100	122	119	96	108	104	105	96	78	75	73	89	95
S.D.		22	39	41	25	33	11	10	11	13	15	11	4	43
TS (%)														
Mean		31	38	35	29	33	31	29	27	23	24	22	28	27
S.D.		9	14	15	10	13	7	3	5	4	5	3	4	15
Normal females	4													
SI (μg/dl)														
Mean		85	102	111	88	70	68	69	65	65	65	75	79	84
S.D.		12	13	10	8	6	4	8	3	5	6	6	8	7
TS (%)														
Mean		26	29	27	20	16	18	19	16	17	20	21	19	20
S.D.		12	13	10	8	6	4	8	3	5	6	6	8	7

Adapted from [60].

large in 2 patients with homozygous HH [156]. Therefore, the variability in normal and homozygous HH individuals is large enough that prudent management usually requires observing more than one set of abnormal SI, TIBC, and TS values before proceeding with additional evaluation. The initial values could be obtained at any time of day. If the initial values are elevated (above 55% for most methods), a subsequent fasting sample should be analyzed.

Interpretation of TS results has been adequately studied in homozygous HH patients, their relatives, and random populations [35,126,128–130,157]. All large studies of homozygous HH patients and relatives confirm that the most sensitive single test for identification of homozygous HH is TS [126,128,129]. Borwein et al. [128] and Bassett et al. [129] used TS decision values of 55 and 50%, respectively, and reported increased sensitivity for detection of homozygous HH if both TS and serum ferritin (SF) were measured. Bassett [129] reported that TS above 50% had sensitivity of 0.82 for homozygous HH, which increased to 0.94 if SF was above 200 ng/ml (200 μg/l) for men or 150 ng/ml (150 μg/l) for women. In Borwein's study [128], TS above 75% and SF above 90th percentile confirmed homozygous HH and TS below 55% and ferritin below 90th percentile excluded homozygous HH. Dadone et al. [126] reported a TS decision level of 62% correctly predicted homozygous HH in 92% of cases whereas SF alone was only 71% correct.

Skikne and Cook [130] reported UIBC alone had high predictive value when testing 40 normal controls and 17 individuals with homozygous HH. He also quotes his study of iron and UIBC in 2829 healthy adults between ages 18 and 64 showing only 1% had low UIBC, suggesting the possibility of good specificity if UIBC were used to screen for homozygous HH. No extensive trial using UIBC alone has been published. Gambino [117] has reported extensive recent reference laboratory experience using UIBC with SI to calculate TS, but clinical follow-up was not reported.

TS has been used in 3 large population screening studies [35,129]. Olsson et al. [157] studied 10 512 samples from 4098 inpatients, 3340 outpatients, and 1311 blood donors during a 2-month period. They reported 1.7% of samples with TS above 70%. Subjects were studied further if the high TS was unexplainable from previous patient data. The prevalence of homozygous HH was 0.24% ($n = 17$) in the population and 11.5% in subjects with TS above 70%. In others, the high TS was due to physiologic iron fluctuations in 44%, liver disease in 22%, blood disorders in 10%, and iron therapy in 10.5%. Among 1311 blood donors, 9 had initial TS above 70% and 3 of these were homozygous HH, but 6 were not. Among 3340 outpatients, 68 patients had TS above 70% in which 11 of these were homozygous HH, but 57 were not. Nearly 50% of the high TS were not high when repeated on subsequent samples following a fast. This obviated further work-up of many patients.

Fourteen patients with repeatable high TS underwent additional follow-up with liver biopsy in 13. All patients undergoing liver biopsy were shown to have homozygous HH.

The second study [35] of 11 065 blood donors used an initial TS decision level of 50% and attempted to follow the 688 high values with a second fasted sample using a TS decision level of 62%. Second TS values were obtained in 465 (68%). For the purposes of this discussion, the untested subjects have been apportioned similarly to those tested. The initial testing for TS above 50% yielded 630 subjects who subsequently had fasting TS below 62% and 58 who were above 62% TS. Liver biopsy showed 69% of these 58 subjects had marked iron overload consistent with homozygous HH and 31% had moderate increases in iron consistent with heterozygosity. No normal individuals were biopsied. False negatives also occurred; the initial TS test was below 62% in 23 of the 58 cases that showed TS above 62% when fasted. Additional family members also benefited; of 102 siblings studied, 23 were found to have homozygous HH.

The third population study included 704 male and 627 female unselected employees [129]. TS exceeded 60% in 5 males and 6 females. Liver biopsy revealed hemochromatosis in 4 individuals; 2 males aged 34 and 39 years and 2 females aged 24 and 43 years. The follow-up was incomplete in the remaining 7 individuals with TS over 60%. This study reinforces the prevalence of hemochromatosis and the ability to identify the disease at an early age in both males and females through unselected testing using TS.

Various decision levels for TS were used in the studies quoted in this practice parameter. The variability between laboratories has also been discussed. It is practical to suggest using an easily remembered TS decision level of 60% unless specific local data identify a specific local decision level. Incorporation of SI, TIBC, and TS in routine chemistry profiles used for general metabolic screening will identify individuals deserving further study.

Normal TS results on 2 occasions in early adult life should exclude HH. The TS may be falsely normal in a hemochromatotic individual with chronic blood loss or inflammatory disease (acute phase reaction). Patients with fasting TS repeatedly above 60% require additional testing for homozygous HH. Homozygous HH has variable clinical course [79,127]. Screening programs tend to find a preponderance of the more slowly progressing cases (referred to as 'length bias') [158]. The benefits to patients and pedigrees from finding even the cases with lesser aggressive expression is expected to be an increase in years of quality life and reduction in morbidity in the tested population.

Phatak et al. [159] have recently presented a computer simulation showing the societal cost of treating presenting symptomatic cases is higher than for screening and prevention for a population of 30-year-old males. They assumed a prevalence of hemochromatosis of 3 per 1000, a test cost of $12, a 40%

chance of developing symptoms of homozygotes, and a discount rate of 3% on future values. The model used in analysis is similar to that proposed in this practice guideline and showed that 70% of all individuals testing repeatably positive would be true positives. The cost analysis of Phatak et al. included testing costs, treatment costs, and costs of later treatment of undetected cases.

A sensitivity analysis for the various parameters in the cost analysis of Phatak et al. revealed that although the cost of screening was less costly than symptomatic treatment if the prevalence were 3 per 1000, a cost of $6921 per life year saved would be incurred if the prevalence were as low as 1 per 1000. As stated earlier virtually all estimates of prevalence among Caucasians are above 3 per 1000.

Screening was favored if the probability of symptomatic disease among homozygotes was 40%. Certainly there are different probabilities of symptomatic disease among different kindreds [32]. However, recent data [79,160] indicate that iron overload with symptoms was highly likely among homozygous individuals found by screening relatives of known cases. In fact, 48 of 51 relatives studied by Powell et al. [79] developed overload. Edwards at al. estimated the prevalence of symptomatic disease in a general population by combining the prevalence of genotypic homozygosity and likelihood of disease development to yield 2.5 symptomatic cases per 1000 males and 0.6 symptomatic cases per 1000 females [160]. Both the Powell et al. and Edwards et al. studies suffer from the potential for selection bias. That is, the individuals tested and used to estimate the frequency of symptom development were relatives of probands. Some probands were identified on the basis of sypmptomatic disease. Therefore, it is possible that kindreds with more aggressive disease may have been more likely to be selected by this mechanism. However, to date there has been no study of a screened population to determine the likelihood of symptoms.

The analysis of Phatak et al. showed that screening was the preferred strategy if the initial test was assumed to have a sensitivity of 96% and a specificity of 94%. However, if these sensitivities and specificities were both reduced to 70%, the cost per life years saved was $4676. Phatak et al. further assumed that the follow-up testing had a sensitivity of 83 and 98%. If the sensitivity were reduced to 60% and the specificity to 70% for the follow-up test then the cost per life year saved would be $5411. Phatak et al. found that the screening strategy was less costly if the test cost $12 or less. If the costs were increased to $20, a year of life saved would cost $1658. Phatak et al. did not carefully differentiate between cost and charge [161]. Charge for test is frequently a poor proxy for the incremental direct resources consumed [162]. If TS derived from automated SI and UIBC were added to routine chemistry profiles the incremental costs are likely to be small. SF requires im-

munoassay. Therefore, in most laboratories the resources required for SF are greater than those for SI and TIBC or UIBC. Automation is becoming more prevalent for SF which will hopefully reduce costs in the future. We feel it is likely that the cost per test of $12 required to prefer the screening strategy is an overestimation of true direct costs. Phatak et al. used discount rate on future values of 3%. This committee will not attempt to predict the future inflation rate.

Buffone and Beck [163] presented a less extensive but similar analysis of the costs and benefits of testing for early diagnosis of HH. They assumed a prevalence of 1 in 300, a likelihood of symptoms in males of 40% sensitivity of the TS tests of 92%, specificity of the TS tests of 98%, a cost of the transferrin test of $10.50, and a cost of the serum ferritin of $13.50. These investigators observed a cost of $605 per year of life saved. Buffone and Beck also considered screening males only.

To date no randomized clinical trial has been published to confirm or refute the models proposed by Phatak et al., Buffone and Beck, and this parameter. However, a study of 12 258 consecutive patients at the Mayo Clinic is in press [164]. These investigators used automated SI and a decision point of 180 μg/dl. They identified 127 individuals with iron above 180 μg/l (32 μmol/l) as the initial test. These individuals were retested with decision points of 180 μg/dl SI, TS of 62%, and SF of 400 μg/l. Forty-four were repeatedly positive by laboratory testing. Thirty of these individuals had a hematologic reason for their abnormal iron studies and 4 had chronic liver disease other than hemochromatosis. Of the 7 remaining cases 3 were homozygous HH, 2 were suspected of being heterozygotes, 1 had another cause of overload and 2 refused workup. The cost of this testing was approximately $33 000. The cost per identified case then would be approximately $11 000. These investigators did not discuss the benefits accrued. In a similar smaller unpublished study in an Iowa community hospital, 819 consecutive outpatients and wellness participants were evaluated [165]. The automated UIBC reagents cost $0.03 per sample. Of the 819, 14 (1.7%) were shown to have an UIBC less than 125 μg/dl (22 μmol/l), indicating a high likelihood of elevated TS. Among these 14 individuals, 5 were shown to have a TS above 50% and a ferritin above the 90th percentile. Among these 5 individuals, 1 was a known sideroblastic anemia, 1 became a new diagnosis of hemochromatosis, 2 were males in their 40s whose serum ferritin had been stable between 350 and 425 μg/l for 4 years (are these heterozygotes?), and 1 individual with a 624 μg/l SF and AST of 51 IU/l has been lost to follow-up. These consecutive patient cohorts at Mayo and Iowa illustrate the low cost of identifying previously undiagnosed individuals. We believe that the laboratory and medical communities should be aware of this low cost of detection of HH. Coupling the low cost with the benefits of increased survival with early detection indicate that a case-finding program should be undertaken.

Sackett et al. [166] have indicated 6 principles that must be satisfied before it is considered beneficial to make a early diagnosis of a potentially fatal disease. First, it must be shown that early detection improves clinical outcome. We believe the clinical outcome of hemochromatosis has unequivocally been shown to be improved by early detection. Even with the caveats of length bias and lead time bias, the data suggest that healthy life years will be increased by early detection of HH. Second, adequate resources to deliver care must be available. The diagnosis of HH is easily made and current medical personnel have the abilities and time to manage these cases. Phlebotomy therapy is inexpensive and available at blood centers and hospitals as well as physicians' offices. Third, compliance with therapy must be acceptable. To date we are aware of no study illustrating the compliance of HH patients identified through case finding or screening. Fourth, a previous demonstration of efficacy should be available. None has yet appeared. However, the models of Phatak et al and Buffone and Beck and the experience at Mayo and Iowa are compelling. Fifth, the burden of disability must be high. Certainly the burden is high for the homozygous individuals who are treated late. And last, the test cost must be acceptable and accuracy acceptable. The test cost can certainly be low if automated UIBC is used as the first screening test. The accuracy of these tests as modeled by Phatak et al. suggest that 2 out of 3 individuals who complete the initial and repeat laboratory testing protocol will be true positives. Eddy states that any health care intervention should show 'actual benefit' and the 'criterion of potential benefit' is inadequate supportive evidence [167]. We believe the quantitative reasoning and careful consideration of costs presented herein indicate testing asymptomatic individuals is appropriate.

A vigorous testing program to prevent the morbidity and premature mortality of undiagnosed HH must also be cognizant of the cost and risks of any false positives. If 2 elevations of TS are observed on fasting serum samples, the specificity of the screening activity will be increased and the false positive rate is expected to be very small. In cases in which the second SI and TS measurements are in the normal range, and the explanation for the discrepancy is not apparent, a third fasting specimen for measurement of SI and TS may be needed. If the algorithm prescribed in Table 11 is followed and the as yet undetected subject with HH has had an opportunity to accumulate iron, an elevated SF will further decrease the likelihood of a false positive. The liver biopsy is useful to quantitate liver iron stores and assess connective tissue morphology. When Edwards followed such a strategy with 11 065 blood donors, those biopsied included 69% homozygous HH, 31% heterozygous HH, and 0% normal individuals. When Olsson followed such a strategy, those biopsied included 100% homozygous HH and also 0% normals. Following the algorithm in Table 11 should provide effective case finding to prevent unneeded morbidity and mortality without risking liver biopsy in any sizable number of normal individuals.

When liver biopsy is contraindicated or refused, estimation of stores by calculating iron removed by phlebotomy confirms the diagnosis of HH. Erythrocyte basic isoferritin (RBC-F) is not as definitive as liver biopsy or estimation of stores by phlebotomy, but may offer a safe, non-invasive alternative with some diagnostic benefit at relatively low cost. The utility of RBC-F for the diagnosis of HH is under investigation [168–170]. All 3 reports show some overlap of RBC-F values obtained from patients with homozygous HH with values from patients with other liver diseases. Furthermore, RBC-F is increased in red cells of patients with hematologic abnormalities associated with poor iron utilization [168]. However, a significantly elevated RBC-F (above 200 attograms/cell) in the absence of hematologic disease is highly predictive of homozygous HH. Patients may have indications for liver biopsy beyond the need to exclude or confirm homozygous HH. Therefore, it will be rare that normal RBC-F will be sufficiently predictive to obviate the need for liver biopsy. However, RBC-F may be useful if liver biopsy is contraindicated or refused.

3.1. Confounding variables

Tests of iron metabolism (iron, TS, SF, and RBC-F) are altered by factors other than homozygous HH or iron deficiency. Hematologic disease alters RBC-F as discussed above. Table 22 illustrates variables that may lead to erroneous interpretations [171–174].

The duration of the increased SI following ingestion of simple ferrous salts is at least 8 h but has not been studied for longer times [136,174]. The amplitude of the increase is greater with greater doses [174] and greater in iron-deficient than iron replete subjects [136,174]. The increase may be as great as 100 μg/dl (18 μmol/l) following a 20-mg dose in iron-deficients [174] and as great as 175 μg/dl (31 μmol/l) in normal subjects following a 176-mg dose [174]. Iron is absorbed most efficiently in the duodenum and less efficiently elsewhere in the intestine. Therefore, the pharmaceutical formulation and dose may alter the duration and amplitude or the increase in measured SI.

4. Histological evaluation in hemochromatosis

4.1. Introduction

Histological interpretation of liver tissue plays a pivotal role in the diagnosis and management of hemochromatosis. The key to this important task is the recognition and quantitation of iron in the form of hemosiderin in liver and in a variety of other tissues including pancreas, myocardium, synovium,

Table 22
Confounding variables in iron testing

Variable	Iron	TIBC	TS	Ferritin
Day-to-day variation	Large	Small	Follows iron	Small
Menstrual cycle	Lower with menses Higher premenstrually	Small	Follows iron	Unknown
Oral iron	Increase after each dose as much as 100–300 mg/dl	No change	Increase after each dose	Increase after many days
Parenteral iron	Increase	Method-specific, may alter results for weeks		
Iron contamination of sample	Increase	No change	Increase	No change
Hepatitis (viral, alcoholic, other)	Increase	Increase in some methods	Increase	Increase
Inflammation/acute phase reaction	Decrease	Decrease	Decrease	Increase
Oral contraceptives	Increase	No change	Increase	No change
Biological variation	Large (20%)	Small (5%)	Large (20%)	Small (10%)
Sample hemolysis	Method-specific, may alter results of stored samples. Marked hemolysis causes spurious elevation of SI and TS			

gastrointestinal mucosa, and several endocrine organs. The diagnosis may be suspected beforehand, and the biopsy may be done with the expressed purpose of confirming the diagnosis and assessing the stage of the disease. In such circumstances, it may be advisable to obtain tissue in a form suitable for the chemical measurement of iron, but this should not be done at the expense of obtaining sufficient tissue to allow for accurate histologic analysis of the liver biopsy. Although small fragments may allow one to assess for hemochromatosis, a minimum length of 1.5 cm is recommended to properly evaluate for architectural changes (assess the biopsy for the degree and character of fibrosis and for the presence of regenerative nodules). Not infre-

quently, the clinical impression of hemochromatosis is erroneous, and the patient with strikingly abnormal elevations of SI studies proves to be a covert alcoholic with overt changes of alcohol-related liver disease on biopsy and little iron present in tissue. In fact, this disparity between the serum and tissue iron is characteristic of liver disease with active necrosis, although it is encountered particularly frequently in active alcohol-related liver disease. To a lesser degree, patients with chronic viral hepatitis [175] and other necroinflammatory diseases of the liver may also present this confusing picture.

Another frequent circumstance is that wherein the recognition of iron occurs only after the biopsy is viewed for the first time. The presence of iron may be recognized in standard H&E sections of liver if its presence is specifically sought; confirmation and grading require an iron stain. Granular brown pigment in periportal hepatocytes (either confined to that location, or with a clear predominance in periportal areas) is iron until proven otherwise. This location is quite reliable in distinguishing hemosiderin from lipofuscin, which invariably is found predominantly in centrilobular hepatocytes. The granules of hemosiderin are larger, more angulated, and more refractile than those of lipofuscin. Recognition of these pigments is further enhanced by careful attention to lighting. The microscope's substage condenser should be dropped just below that considered to be ideal by Köhler illumination, and the iris diaphragm should be closed down slightly to give added contrast. This maneuver should ensure that significant amounts of iron are not missed in viewing standard sections. Although an experienced observer will routinely recognize significant amounts of hemosiderin in routine H&E sections, an iron stain will ensure that significant iron problems are not missed.

The semi-quantitative estimate of the magnitude of iron deposition in biopsy and autopsy liver sections remains the cornerstone of the pathologist's contribution to the diagnosis of hemochromatosis, particularly in the screening situation. Iron is readily recognized in H&E sections (at least when present in significant amounts). An iron stain should be performed so that quantitation can be done. The stain of choice is the Perls' stain because of its sensitivity and specificity [176]. Ferritin is seen as a diffuse blue tint to the cytoplasm, while hemosiderin is visualized as dense dark blue granules.

There are a number of grading systems in use, but the one described by Scheuer et al. in 1962 remains the most widely used [177]. Its advantage is that it is easily and rapidly applied, and deals only with hepatocyte iron. Its disadvantage is that it is somewhat subjective, its application depending on a comparison with 4 color photographs in the 1962 article (no clearly defined verbal description is given in that source). Other systems such as those put forth by Brissot et al. [95] and Deugnier et al. [178,179] are perhaps more accurate but are more difficult to apply. There is reasonable but imperfect

Table 23
Graping of hepatic iron content

Scheuer grade	Gross appearance of slide[a]	Microscopic appearance
0	Pink to red	No blue granules (at 100X or 450X magnification)
1	Pink to red	Blue granules in less than 5% of hepatocytes or diffuse faint blue (at high magnification)
2	Faint purple	Blue granules present in 5–10% of hepatocytes (periportal)
3	Purple	Abundant blue granules present in up to 40% of hepatocytes (periportal accentuation with central sparing)
4	Deep blue	Abundant blue granules in more than 40% hepatocytes (decreased load centrilobularly)

[a]The actual grade is assigned on the basis of the microscopic appearance alone [156].

correlation of the various grading systems with chemical analysis. It has been demonstrated that the Brissot et al. systems correlate better with chemical analysis than the Scheuer et al. system. This probably reflects the fact that it includes non-hepatocyte iron. It must be remembered that it is the excessive hepatocyte iron, at least in the early stages, that characterizes hemochromatosis. It should also be stressed that chemical analysis and histologic grading are complementary and the maximum information is available when the results of each are carefully correlated. The task force recommends a modification of the Scheuer et al. system [14,156] as depicted in Table 23 and Fig. 1.

From the standpoint of the role the morphologic pathologist plays, recognition of large amounts of iron (3 or 4 plus in the modified Scheuer et al. system) must be considered evidence for hemochromatosis until there is evidence to the contrary.

While there is some evidence that image analysis improves the ability to grade the degree of hemosiderin deposition in liver [101] the equipment for such analysis is not widely available and this added step is unnecessary.

Attention must also be paid to the location of hepatic iron. Hemosiderin that is present solely or predominantly in the Kupffer cells is not indicative of hemochromatosis. It likely comes from transfusions, intramuscular injections of iron, hemolysis, or alterations in metabolism of iron associated with

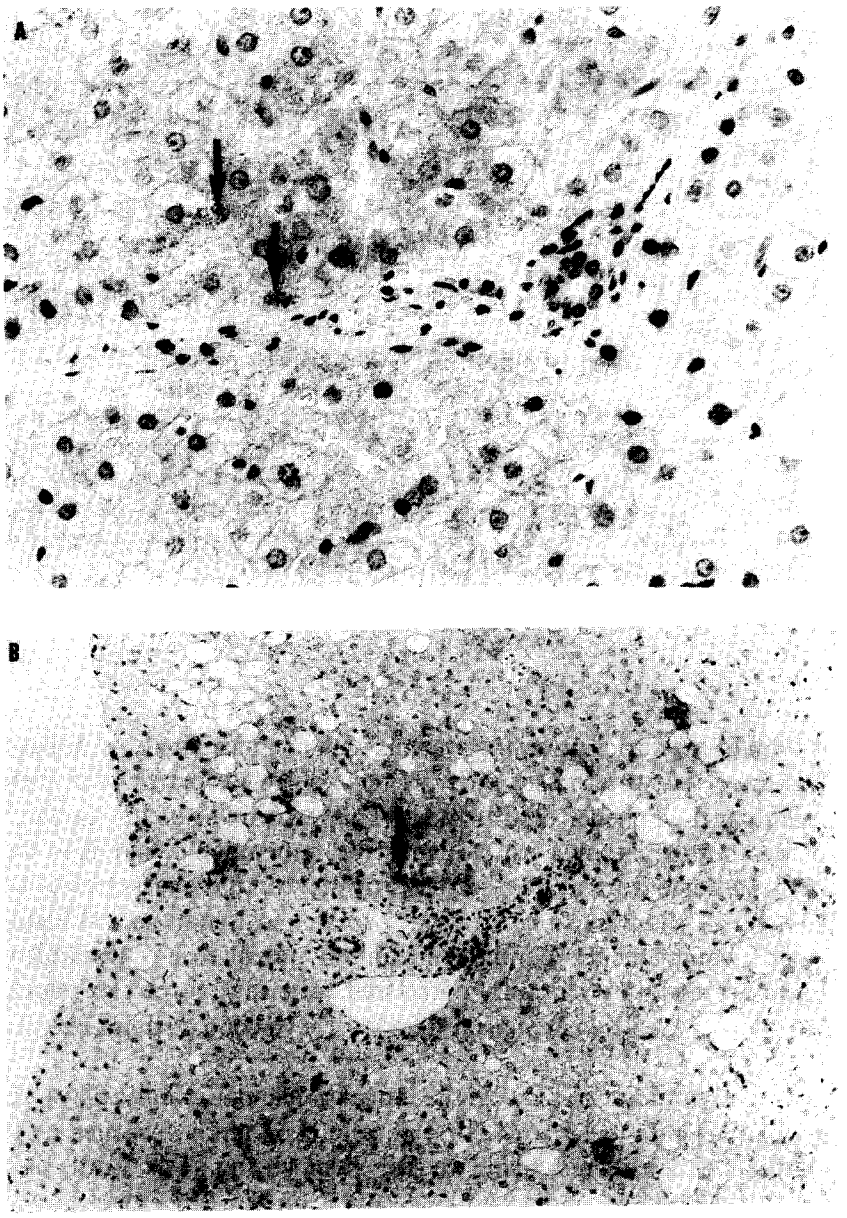
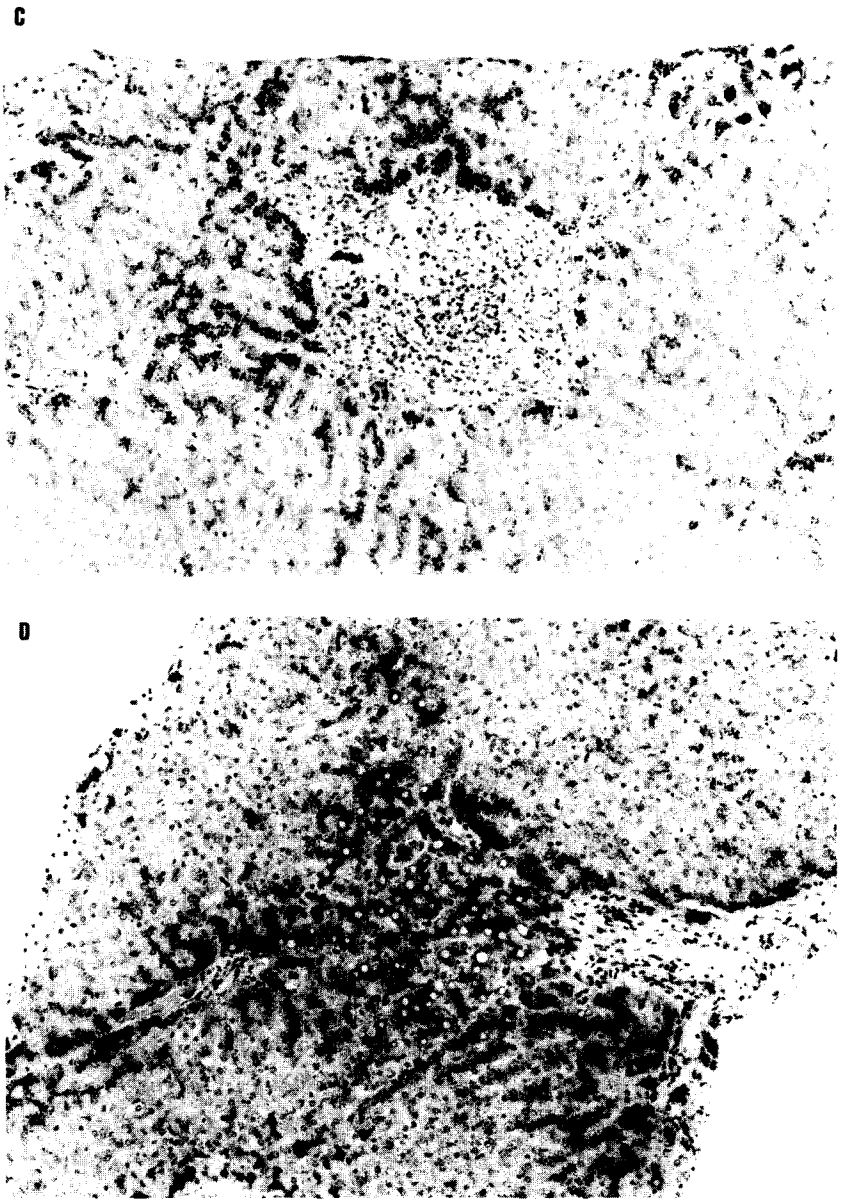


Fig. 1. (A) Grade 1. Hemosiderin granules are present in only several hepatocytes (arrows); these granules stain deep blue and are readily visible against the pale pink background of Perls' stained glass slides (Perls, × 100). (B) Grade 2. Hemosiderin granules are present in less than 10% of hepatocytes (arrow) (Perls, × 33). (C) Grade 3. Hemosiderin granules in about 25% of hepatocytes. There is a clear tendency to involve periportal hepatocytes. This is a fairly early grade 3. With grade 3, hemochromatosis becomes a strong consideration (Perls, × 33). (D) Grade 4. There are dense hemosiderin granules in about 50% of the hepatocytes. The periportal preponderance is maintained (Perls, × 33).



chronic illness. In longstanding hemochromatosis, probably as a result of hepatocyte necrosis, iron begins to appear in the Kupffer cells. Likewise, patients with severe transfusion overload begin to accumulate iron in their hepatocytes. The mixed hepatocyte reticuloendothelial pattern of hemosiderin deposition is ambiguous and requires further study for clarification.

Fig. 2. The enlarged portal triads link to encircle regenerative nodules. The triangular extensions of collagen resemble the contours of a holly leaf (Masson, ×10).

Iron can appear in the connective tissue in hemochromatosis, but can also appear there in such circumstances as multiple transfusions, dialysis, and Bantu siderosis. One helpful clue is the character of the fibrosis in hemochromatosis, which is primarily portal and periportal and resembles that seen in biliary disease. It has imaginatively been described as having a 'holly leaf' appearance (Fig. 2) [180].

The cirrhosis that may result usually has a micronodular appearance. Iron in bile duct epithelium has been considered by some as a specific marker for genetic hemochromatosis, but is not a reliable marker in this regard. Not all cases of hemochromatosis result in biliary hemosiderin deposition, and duct hemosiderosis can be seen in such circumstances as transfusional overload. It has been shown in the patient with hemochromatosis that fibrosis appears to be related to the deposition of iron within the portal structures, including bile ducts. Likewise, the presence of necrosis of hemosiderin-laden hepatocytes (sideronecrosis) correlates well with the presence of progressive necrosis [178].

Small foci of hepatocytes that are devoid of hemosiderin are present in a minority of patients with hemochromatosis. They may represent areas of regeneration, although on occasion they may show features suggestive of dysplasia [178].

4.1.1. Chemical analysis of liver tissue

The chemical analysis of iron in tissue is useful in evaluating ambiguous cases. Liver is the only tissue so analyzed with regularity in clinical practice. Methods and results may vary depending on where the analysis is performed: careful attention must be paid to the ranges and interpretations by various laboratories. It must be remembered that liver iron concentration normally increases with age. In general, values in excess of 300 μmol/g are considered to be strong evidence for genetic hemochromatosis if there is no other obvious reason for the iron accumulation (such as multiple transfusions or other parenteral sources) [175]. Asymptomatic homozygotes may have considerably lower levels, depending on their age at the time of diagnosis. Architectural damage is not observed at levels below 250 μmol/g in the absence of alcohol abuse [103]. Such factors as alcoholism, sex, and age reduce the iron overload threshold necessary to cause fibrosis [181]. While most of the literature deals with analysis of fresh liver tissue, it is not necessary that the analysis be performed in this way (see Tables 12–14). Chemical analysis of standard paraffin embedded material can readily he done (a piece of the standard needle biopsy of liver between 3–5 mm is ideal) [176]; a good correlation with the analysis of properly handled freshly obtained tissue has been shown. This obviates the need for special handling of the liver biopsy specimen, and ensures that the quantitative iron value is interpreted in the context of a histologically analyzed specimen. This allows for recognition of unexpected diseases, allows one to determine the distribution of iron in the liver, and provides the maximum specimen for evaluation of the degree of fibrosis. The utility of quantitating iron can be further enhanced by quantitating the Hepatic Iron Index (HII) [175]. The HII (iron in μmol/g dry weight of liver divided by the patient's age in years) aids in distinguishing HH from other causes of iron overload (such as alcohol-related liver disease) (see Table 13). Values of HII 2.0 or greater separated individuals with homozygous hemochromatosis from heterozygotes and from alcoholics with secondary iron overload and other liver diseases. This has been documented in several studies and is a more effective discriminator than liver iron concentration alone [98,100–102]. A histological hepatic iron index (HHII) has recently been proposed as a further refinement in evaluating biopsies with iron overload when chemical analysis of tissue cannot be performed [179].

4.2. Iron in extrahepatic sites

4.2.1. Pancreas

While significant amounts of hemosiderin are routinely seen in both acinar and islet cell tissue in hemochromatosis, such deposition can also occur in other situations when there is massive iron overload. In both instances, iron

appears preferentially in acinar tissue. It can also be found in duct epithelium and interstitium, with resulting fibrosis [182].

4.2.2. Myocardium

Hemosiderin is found consistently in the cytoplasm of cardiac muscle cells (rather than the interstitium) in patients with genetic hemochromatosis [183,184]. Even though distribution is somewhat patchy and tends to be most pronounced in the subepicardial region, clinically obtained endomyocardial biopsies may have stainable hemosiderin in genetic hemochromatosis, and a Perls' stain should be considered on specimens from all patients who have such biopsies for cardiomyopathy of unknown etiology.

4.2.3. Synovium

Since hemochromatotic arthropathy may antedate clinical liver disease by many years, one must be aware of the histologic appearances of this disease process. Hemosiderin preferentially located in synovial lining cells (as opposed to connective tissue) is a valuable clue. Bleeding into the joint tends to lead to hemosiderin in macrophages and in connective tissue. Calcium pyrophosphate deposition (pseudogout) is often present in hemochromatotic arthropathy and may provide another clue. Likewise, there may be a characteristic splitting at the interface between articular cartilage and subchondral bone [50].

4.2.4. Gastrointestinal tract

Hemosiderin may be present in the mucosa of the stomach and proximal small intestine in hemochromatosis. The presence of hemosiderin in gastric, duodenal, or jejunal glandular epithelial cells should elicit a careful work-up for hemochromatosis; hemosiderin in lamina propria macrophages is less meaningful, in most instances resulting from absorption of heme from a gastrointestinal bleed [185].

4.2.5. Skin

Skin biopsies taken from an area of abnormal pigmentation in an individual with HH may show several abnormalities of pigmentation. The major contributing factor to the abnormal pigmentation is a disturbance in melanin metabolism, although hemosiderin may also be deposited. The melanin will be present in increased amounts in the basal layer of the epidermis. Hemosiderin may be present both extracellularly and within macrophages, particularly around blood vessels and in the basement membrane of the sweat glands. Iron granules may also be present in the epithelial cells of the sweat glands and occasionally in the basal layer of the epidermis. The iron may stimulate melanocytic activity by increasing oxidative processes or by reacting with sulfhydryl groups in the epidermis.

4.2.6. Other sites

Parenchymal sites throughout the body may be the site of hemosiderin deposition in hemochromatosis. Endocrine organs (thyroid follicular and parafollicular cells, adrenal anterior pituitary) are particularly likely to be involved [186]. The gonadal problems common in hemochromatosis result from the destructive influence of hemosiderin in the anterior pituitary. Bone marrow hemosiderin is not increased in uncomplicated hemochromatosis.

4.3. Summary of histopathology

It should be stressed that there is no one single histological finding or location of iron that is pathognomonic for HH. Instead, it is the presence and amount of hepatocellular hemosiderin, in the absence of an acceptable explanation (transfusion, dialysis, etc.), that is the histologic hallmark of hemochromatosis.

The most common and sometimes confusing clinical situation deserves special mention. Hemochromatosis and alcohol-related liver disease with hemosiderosis can usually be separated. It must be borne in mind that one will encounter patients with hemochromatosis who are alcoholics, and alcoholics with liver disease who prove to have hemochromatosis unexpectedly on biopsy. The key is the quantitation of iron in the biopsy [97]. When typical changes related to alcohol (fat, intrasinusoidal fibrosis, hyaline) are overt and hemosiderin is minimal (trace or 1 plus), the damage is considered solely due to alcohol. If the iron is judged 3 or 4 plus, the patient probably has hemochromatosis; this would appear to be a certainty if the fibrosis is portal and periportal ('holly leaf') rather than central and intrasinusoidal [187]. The relatively unusual circumstance of a 2 plus biopsy is more difficult, and may require a judgment based on all those features discussed throughout this article.

The pathologist should take on a slightly different philosophy than usual with regard to the diagnosis of hemochromatosis. One must pursue this diagnosis aggressively and the possibility should be suggested to the clinical physician anytime there is an unexplained significant deposition of hemosiderin in biopsy tissues. The tragedy of missing a devastating but treatable familial disease far outweighs the small price a patient or family members might pay once the diagnosis is suggested (relatively inexpensive further screening; phlebotomy once the diagnosis seems certain). Both surgical pathology and autopsy pathology play important roles. Each section of liver must be analyzed with the idea in mind that significant hemosiderin deposition must be excluded. If such deposition is found, it must be explained through correlation with clinical information, seeking further histological clues, or obtaining biochemical tests. Clues may turn up unexpectedly in the

surgical pathology laboratory (the hip replacement specimen from a young male, the endomyocardial biopsy, the gastric biopsy, etc.). The autopsy liver may provide a valuable clue to the presence of a genetic disease affecting the patient's survivors. One of the many responsibilities of the pathologist is to communicate these findings to the appropriate person to initiate family screening.

Biopsy is recommended in all patients who are thought to have hemochromatosis. This allows one to confirm the diagnosis and exclude other diagnostic possibilities, and quantitate the degree of iron deposition and the amount of fibrosis (i.e. to stage the disease). Although there are well-established risks with liver biopsy [188–191] (clinically significant hemorrhage in 0.32%, death in 0.01–0.1%), these must be weighed against the need to firmly diagnose treatable liver disease. The risk in hemochromatosis patients is probably less in that most significant complications occur in patients biopsied for malignancy.

In summary, the anatomic pathologist must consider the possibility of hemochromatosis whenever liver tissue is being evaluated. The ideal evaluation includes the following: (1) performance of a Perls' stain for iron on all liver tissue; (2) semiquantitative estimation of degree of iron deposition (modified Scheuer grading). Note: this presumes a distinction has been made between Kupffer cell iron and hepatocyte iron; (3) estimation of the degree of fibrosis. Three categories are recommended: (a) no fibrosis; (b) pre-cirrhotic portal and periportal fibrosis ('holly-leaf' patterns); (c) cirrhosis; (4) evaluation of other organs for iron overload (if these are available in the pathology block and slide files from prior biopsies from patients with liver iron excess); (5) evaluation for coexisting diseases or diseases masquerading as hemochromatosis; (6) consideration of quantitative iron analysis (of paraffin embedded materials) and HII in controversial cases; (7) consideration of the possibility of a complicating hepatocellular carcinoma in cases of established iron overload.

4.4. The phlebotomy program

4.4.1. Frequency of phlebotomy

The rate of phlebotomy must be established for each patient individually. This is done by monitoring the hemoglobin or hematocrit (H/H) *before* each procedure in order to establish and maintain a mild blood-loss anemia. For example, if the hematocrit before any phlebotomies is 45%, maintaining it at 40% provides an adequate challenge to the marrow without provoking symptoms of anemia. Holding the hematocrit at 30% or even 35% does not increase marrow output, and the patient may have symptoms of anemia. Furthermore, each phlebotomy recovers less iron (1 ml RBC contains 1 mg

iron.) Some patients feel 'improved' just after a phlebotomy while others feel fatigued and prefer the procedure on Friday afternoon, allowing Saturday to rest which limits weekly phlebotomies to one. Many patients tolerate more than 1 per week. During the first 6–8 weeks, the erythropoietic marrow usually responds to the challenge of mild anemia by increasing its output of RBC, which allows the rate to be increased [192,193].

4.4.2. Difficult access

Patients with cool hands have contracted arm veins. They should soak both hands in very hot water for 5 min just before phlebotomy. Hot compresses don't work. Some patients without adequate superficial veins require installation of a central line. A subcutaneous port is recommended. In obese patients the surgeon should tack the port to the dermis.

4.4.3. Size of phlebotomy

When phlebotomy is performed at blood banks, the size tends to be trimmed to their procrustean regulations, i.e. 450 g, even though the blood is not used for transfusion. Small, debilitated, and elderly patients may require removal of smaller volumes. On the other hand, robust patients can tolerate the loss of a larger-than-standard volume; the bag can be filled to its right capacity, about 600 g, thus recovering an additional 50 mg iron each time. With time and experience some patients tend to become tolerant of blood loss, thus permitting the volume to be increased [192,193]. Other patients may require the volume to be reduced. It is a good practice to urge patients to 'fill up' with fluids just before coming to phlebotomy; to drink at least 2 tumblers of fluid during the preceding hour.

4.4.4. Who should be treated?

The demonstration of increased stores of iron — not waiting for iron-storage disease to develop — is the indication for removal of the iron. Age itself should not be a restriction: the 60-year-olds may have parents in their 90s. Of course, in the presence of hepatocellular carcinoma or other terminal illness, common sense must prevail. Heavy iron loading is exceptional in childhood, but it does occur. Siderotic heart disease and menopause (pituitary siderosis) have occurred in teenagers indicating an early onset of critical accumulation of iron [194]. When family screening tests are positive in youngsters, the severity of iron loading should be tracked by serum ferritin.

4.4.5. Prediction of required number of phlebotomies

The elevation of the SF can provide a rough clue concerning the number of phlebotomies needed to produce iron deficiency when the values are at the lower end of the scale [195]. Ferritin 500 m/l usually requires less than 10

phlebotomies. Values of 500–1000 require less than 25. But values above 2500 require 'a whole lot.' Some clinical laboratories report high values as >500 or >1000. To obtain true values, such sera should be diluted to × 10.

4.4.6. Alternative and adjuvant programs

4.4.6.1. Diet during phlebotomy therapy. Should the diet be changed in any way? With 2 or more phlebotomies a week, additional dietary protein may be needed to compensate for the loss of hemoglobin. (Hemoglobin is 96% protein. The loss of plasma protein is so small as to be insignificant: plasma is 6% protein.) No effort should be made to avoid iron-containing foods, or vitamin C which facilitates absorption of iron. Nor need it be recommended to drink tea with meals because it impedes absorption of iron. Such adjustments of diet are unnecessary. The maximum amount of dietary iron that can be absorbed per day is quite small (ca. 5 mg) compared with the amount removed with every phlebotomy (200–300 mg).

It is recommended *not* to take vitamin-mineral dietary supplements that contain iron. It is emphatically recommended not to use alcohol. One hepatotoxin at a time!

4.4.6.2. Chelation therapy. Chelating agents to remove storage iron do not provide a reasonable way to treat hemochromatosis. Chelation is clumsy, uncomfortable, and expensive [196]. Phlebotomy is cheap, quick, and more efficacious.

Oral chelators, which can be absorbed and increase iron excretion, are being used experimentally [197].

4.5. Response to phlebotomy therapy

4.5.1. The design of phlebotomy therapy

The aim of therapy is to remove the pathogenic iron as rapidly as possible while minimizing risk to the patient. The rate at which hemoglobin is removed can be adjusted by the frequency of phlebotomy and by the volume of blood removed each time. The blood bag is weighed before and after the procedure.

Hemoglobin or hematocrit and the amount of blood removed are the only parameters measured during the phlebotomy program — until the end. The measurement serves 2 functions: (1) by establishing the level of anemia, it determines whether this phlebotomy should be performed or not; (2) together with the volume (weight) of blood removed, the loss of iron can be computed: hemoglobin concentration multiplied by weight gives the grams of hemoglobin. Hemoglobin is approximately 0.35% iron. Thus the iron decrement can be computed. When hematocrit alone is measured, the value divided by 3 approximates the hemoglobin concentration. (Red cells are 33%

hemoglobin.) Each patient's clinical chart should log the date, weight, and hemoglobin concentration for each phlebotomy. The decrement of iron is also computed and recorded. In addition, each patient should be required to maintain his or her own log, showing date, weight of blood removed, and the hemoglobin concentration. This is a valuable record should the patient move; it also increases his or her sense of participation in the program, and it keeps the patient sitting in the waiting room for a few moments while the bag is weighed, thereby reducing the possibility of syncope.

SI and SF need not be monitored during the phlebotomy program because they contribute no information essential to the decision about phlebotomy.

4.5.2. The end game

When hemoglobin is promptly replenished after each phlebotomy, easily mobilized storage iron must be present. When the blood loss anemia is not repaired after phlebotomy, a problem is present: is the storage iron exhausted? Or has an inflammatory disorder impaired erythropoiesis? Even a febrile coryza or the flu can do it. Waiting out an obvious infection can solve the problem: with defervescence the anemia improves. When there is no evidence of a phase reaction, measurement of SI and ferritin are useful, but the results require thoughtful consideration. When hemochromatosis is in a steady state (i.e. no recent blood-loss, no recent phlebotomy) if there is any storage iron at all, even less than normal, the SI is usually elevated [193]. But during a phase reaction the SI, even in untreated hemochromatosis, can be suppressed to a normal level; in this situation, however, TIBC is also suppressed, and the saturation index remains abnormally high [198].

When phlebotomy therapy has removed all storage iron, the usual finding is SF < 20 μg/l. However, serum ferritin, being a phase reactant, is increased in the presence of inflammation. Indeed, when the iron stores are completely empty, inflammation can raise the SF to a normal level [199].

4.5.2.1. Phlebotomy-induced debility. In some middle-aged or elderly patients who are near the final depletion of storage iron, the phlebotomy-induced anemia does not correct itself, and the patient reports unaccustomed fatigue, malaise, syncopal episodes, and emotional depression. Attempts to continue phlebotomy therapy only make the debility worse. Serum ferritin values indicate the presence of storage iron which the challenge of blood-loss anemia has not mobilized. In male patients, depotestosterone may correct the syndrome dramatically. The hemoglobin level may return to normal promptly and the symptoms disappear [200]. This indicates that the elevated SF levels were indicative of storage iron, not a phase reaction. In women with phlebotomy-induced debility, subcutaneous erythropoietin has behaved in the same way to mobilize this 'last iron.'

Why is it important to induce iron deficiency? We do not know what form

or what location of abnormal storage iron is pathogenic. Therefore, it seems prudent in the initial phase of phlebotomy therapy to induce, insofar as possible, the removal of all storage iron, leaving the patient with mild, but absolute, iron deficiency anemia. After initial iron depletion, the hematocrit is allowed to return to normal and remain in the normal range.

4.5.3. SI after phlebotomy therapy

Recovery from phlebotomy-induced iron deficiency anemia proceeds at a rate that requires the daily availability of as much as 8–10 mg iron. When all storage iron is gone, the patient remains mildly anemic. But when this anemia has been repaired by the absorption of dietary iron, the SI often jumps immediately to an abnormally high level [193]. This is a peculiarity of hemochromatosis and it does not mean that iron stores have become rapidly overloaded. Trust the SF result to demonstrate the empty stores.

Moral: Do not be misled by high SI at the moment of recovery from phlebotomy-induced anemia in hemochromatosis. It does not mean overload.

4.5.4. Lifelong phlebotomy schedule

Hereditary hemochromatosis manifests itself clinically by the accumulation of unneeded iron: the diagnosis of hemochromatosis requires the demonstration of this accumulation. Phlebotomy therapy, by inducing negative iron balance, removes the iron. When the therapy is interrupted, positive balance reasserts itself and accumulation of unneeded iron resumes. For this reason phlebotomies must continue — for life. Patients should understand this from the onset of phlebotomy therapy.

The rate of 'maintenance phlebotomy' is established for the individual by determining the rate at which he or she accumulates iron. Bear in mind that, in hemochromatosis, the iron-deficient intestine increases absorption of iron, it responds *in sum* to the signal of hemochromatosis plus the signal of blood-loss anemia [193]. For this reason the patient's requirement for maintenance phlebotomies should be established after the patient is clean of abnormal iron and after the patient has repaired any blood-loss anemia. This is done by measuring the SF at 6-month intervals and performing phlebotomy when the ferritin level is at or near 100 μg/l. Phlebotomies may be needed 2, 4, even 6 times per year. To keep the patient even marginally anemic increases iron absorption and the requirement for phlebotomy. An occasional elderly patient seems not to require maintenance phlebotomy at all. In such cases, however, surveillance should be maintained by means of SF every 6–12 months.

4.6. Monitoring complications of hemochromatosis

The complications of hemochromatosis can be divided in 2 parts: those which may be improved by the removal of storage iron and those which are not. Life expectancy has been improved by phlebotomy therapy of hemochromatosis. Patients who are diagnosed and treated before the clinical appearance of any complications of hemochromatosis have a normal life expectancy [43]. Those with florid complications have a less-than-normal life expectancy, but, even so, it is better by far, than the expectancy of those who elect not to be treated [201]. With regard to the foreshortening of life expectancy, cirrhosis, especially cirrhosis with diabetes, is the most pernicious of the complications [43].

4.6.1. Complications improved by phlebotomy

Heart disease: restrictive cardiomyopathy and refractory cardiac arrhythmia caused by myocardial siderosis with or without the congestive failure are usually improved or completely cured by removal of the stored iron. It is especially urgent that young patients with hemochromatosis be treated to correct or prevent cardiac disorder and death. The young heart is especially susceptible [194].

Abnormal pigmentation of the skin usually disappears.

Diabetes mellitus is improved, and it sometimes disappears during the induction of iron deficiency. The improvement may be temporary.

Liver impairment, manifested by chemical abnormality, subsides during phlebotomy therapy. Hepatomegaly may disappear. In a few cases, which have been carefully monitored and kept iron-free for many years, cirrhosis has disappeared [52]. (One of these patients died of hepatocellular carcinoma 25 years later.)

4.6.2. Complications not improved by phlebotomy

Hepatocellular carcinoma is the leading cause of death among hemochromatosis patients in whom cirrhosis is present, causing 30% of all deaths [43]. Other liver diseases cause an additional 15% (hepatic failure 11%, variceal hemorrhage 4%). Alpha fetoprotein (AFP) is a marker for hepatocellular carcinoma. However, AFP has not yet been demonstrated to be of value in surveillance of patients with hemochromatosis.

Arthropathy is seldom improved by phlebotomy; indeed it often progresses during the course of therapy [50]. Gonadal failure is almost always irreversible. Sexual impotence and the symptoms of premature menopause can, like diabetes, be treated by hormonal manipulation, but the sterility imposed by anterior pituitary failure is permanent [202].

Liver transplant has been performed in a few carefully selected patients who had severe cirrhosis; the procedure is, at present, controversial.

4.6.3. CNS manifestations of hemochromatosis

Oppressive fatigue, together with lethargy, is the commonest sysptom of hemochromatosis [43]. Emotional depression is also common and often a presenting complaint. Some patients have become mentally retarded, confused, obtunded, disoriented, even stuporous [58]. In hemochromatosis there is no abnormal iron accumulation in the CNS except on the choroid plexus [75]. The pathogenesis of the above phenomena must have a complex basis, but the abatement of the symptoms during therapy — which does occur in some patients — indicates their etiology [58].

4.6.4. Survival

Patients who are adequately treated before the onset of diabetes or cirrhosis have a normal life expectancy. Fifteen years after diagnosis, 80% of non-diabetic, non-cirrhotic patients are living, whereas only 55% of cirrhotics and 50% of diabetics have survived [10]. It should be noted that all of the latter 2 groups were treated with appropriate phlebotomy. Without treatment, survival of such patients is further shortened.

5. Counseling

As is evident from the preceding comments, widespread screening and early diagnosis of HH should be followed by an aggressive program of treatment of the iron overload and any of its complications. An integral part of this treatment must be the counseling of newly diagnosed patients as to the implications of diagnosis and treatment, whether dietary or other restrictions should be imposed, and of the need for testing of potentially affected relatives.

5.1. Counseling newly diagnosed patients

(1) Patients with no evidence of organ injury (including absence of cirrhosis) may be reassured that with proper treatment, their lifespans should not be any different from those of their age- and sex-matched contemporaries. Sometimes the arthropathy develops during or following the course of phlebotomy therapy, as may also, in some cases, diabetes mellitus. Absolute abstinence from alcohol consumption should be advised. No other restrictions in diet or activities should be imposed. Specifically, it is not appropriate to counsel reduction in meat or bread intake or otherwise limit the diet beyond that which is prudent for the population generally. For example, the daily ingestion of meat of any kind each day in pound or kilogram quantities is ill-advised for those with hemochromatosis. A diet of moderation is advised. There is no need to advise either increased or decreased consumption of tea or coffee.

(2) Patients with evidence of organ injury include those with cardiomyopathy, cirrhosis of the liver, arthropathy, diabetes mellitus, or gonadal failure. Patients who have evidence of cardiomyopathy alone may be advised that treatment should markedly improve their cardiac function. Those with cirrhosis of the liver, diabetes mellitus, arthropathy or gonadal failure may be advised that these abnormalities are likely to persist and will probably require specific treatment for the affected organ system, but that removal of the iron excess may reduce the severity or rate of progression of these conditions. They should be advised that treatment is likely to extend their lifespans, but that whether this will be so, and to what extent, is uncertain. The same principles should be followed with respect to abstention from alcohol consumption or exposure to other hepatotoxins and little or no dietary restriction as advised for patients without organ damage.

(3) For all patients, a proper diet should provide adequate quantities of all needed vitamins and minerals. Vitamin supplements are not needed. However, patients should be specifically warned that they must not take vitamin supplements 'fortified' with minerals that include iron. Most such preparations that are available without prescription contain 18 mg of iron per pill. One iron-fortified vitamin supplement ('Geritol™') contains 50 mg of iron per pill.

5.2. Counseling family members

Once the diagnosis of hemochromatosis has been made, it is incumbent upon the physician to test other family members in order to identify others who may be affected. Siblings are especially at risk, and they may be advised that each has an approximately 25% probability of also having homozygous hemochromatosis. As a minimum, they should be advised to have a blood test for SI and iron binding capacity and for ferritin. If the results are abnormal, they should be further investigated for hemochromatosis, as outlined earlier. If the results are normal, the test should be repeated at intervals of not less than once every 3 years. HLA testing may be recommended to siblings of affected cases, with the advice that if they are HLA identical, they may be presumed also to be homozygous for HH. While SI, TIBC, and ferritin tests should be recommended for all siblings, parents, and children of index cases, HLA testing is not appropriate for the parents or children of the affected person. This is because even in the unlikely event that the patient and child (or parent) are HLA identical, the child cannot inherit both hemochromatosis genes from one parent. Furthermore, HLA testing of siblings should be done only once in any family and usually is not needed at all.

5.3. Behavioral guidelines

These have been discussed above in Section 5.1. Briefly, patients should

be told that they must practice absolute abstinence from alcohol for the rest of their lives (except as noted above for patients without evidence of organ injury), and that other dietary or behavioral restrictions are not usually needed. They must, however, complete the phlebotomy program and have periodic examinations and phlebotomies to reduce iron loading in the future.

All patients with iron overload should be advised to abstain from contact with uncooked marine seafoods, especially shellfish, in view of the risk of sepsis due to *Vibrio vulnificus* in iron overload disease.

Some patients may be engaged in occupations or hobbies that expose them to other hepatotoxins such as cleaning fluids or other organic solvents. These must be avoided.

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