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Clinical practice guidelines on hemochromatosis: Asian Pacific Association for the Study of the Liver

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Introduction and summary

Hereditary hemochromatosis is the result of pathogenic variants in multiple genes that can result in increased body iron stores with excess iron deposited in various organs, including the liver, pancreas, and heart. The two most important advances in the field over the past 30 years have been the identification of the HFE gene (and the associated p.Cys282Tyr substitution), and the discovery of the hormone hepcidin, which is inappropriately low in this condition and is the pathophysiological basis of the increased iron absorption. The identification of mutations in the HFE gene and subsequent studies have reshaped diagnostic algorithms resulting in a marked reduction in the need for liver biopsy. The discovery of hepcidin has resulted in many studies that have dramatically improved our understanding of iron metabolism with clear potential therapeutic implications.

The variable clinical expression of hemochromatosis has puzzled clinicians and scientists, and our understanding of the factors that influence the phenotype has increased over recent years. Nevertheless, increased clinician and patient awareness, early diagnosis, and therapeutic phlebotomy to restore normal life expectancy are still the cornerstones of management. The classic triad of cirrhosis, diabetes, and skin pigmentation is now uncommon, and many patients are diagnosed with minimal or no symptoms.

These guidelines have been developed to assist clinicians in the management of patients with hemochromatosis. They have been developed with the recent passing of Professor Lawrie Powell fresh in our minds. Professor Powell was one of the world's leading authorities in the field of iron metabolism and hemochromatosis, and a co-founder of the Asian Pacific Association for the Study of the Liver. The authors dedicate these guidelines to the memory of Professor Powell in recognition of his remarkable contribution to knowledge in the field.

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Overview of iron metabolism

The total body iron pool of a normal adult is approximately 3–4 g, or 50 mg/kg. Most of this iron is located in erythrocytes as hemoglobin (60–70%) with the balance located in skeletal muscle, liver, spleen, and bone marrow as storage iron in ferritin and hemosiderin, iron-containing enzymes, and bound to transferrin. The average Western diet contains approximately 6 mg of iron per 1000 cal. Of this, only about 1–2 mg per day is absorbed by the duodenal mucosa.

Iron absorption occurs in the enterocytes of the small intestine (predominately the duodenum and first section of the jejunum). Heme iron absorption is thought to occur via endocytosis, but little is known of this process [1]. Divalent metal transporter 1 (DMT1) is responsible for the uptake of ferrous iron (Fe²⁺) following reduction of ferric iron (Fe³⁺) by duodenal cytochrome B (DCYTB). Excess iron is then

stored in ferritin or is lost through enterocyte sloughing. Ferrous iron is exported through ferroportin (FPN) on the basolateral membrane of the enterocyte and oxidized by hephaestin (Fig. 1). Approximately 80–85% of absorbed iron is transported by transferrin to the reticuloendothelial system or to bone marrow for incorporation into hemoglobin, stored in the liver or muscle, or used for heme synthesis [2].

The liver is an important organ in iron metabolism through its storage of iron and its synthesis of transferrin and hepcidin. The iron-sensing molecule, hepcidin, is responsible for sensing body iron stores and acts as a negative regulator. Increased iron levels result in increased hepcidin synthesis, which decreases release of iron into the circulation. Hepcidin binds to the iron export protein, ferroportin, on target cells and is responsible for the internalization and degradation of ferroportin. Patients with hereditary hemochromatosis have inappropriately reduced hepcidin expression due to mutations in *HFE* [3]. In addition to iron, infection, inflammation, and hypoxia can alter hepcidin expression [4].

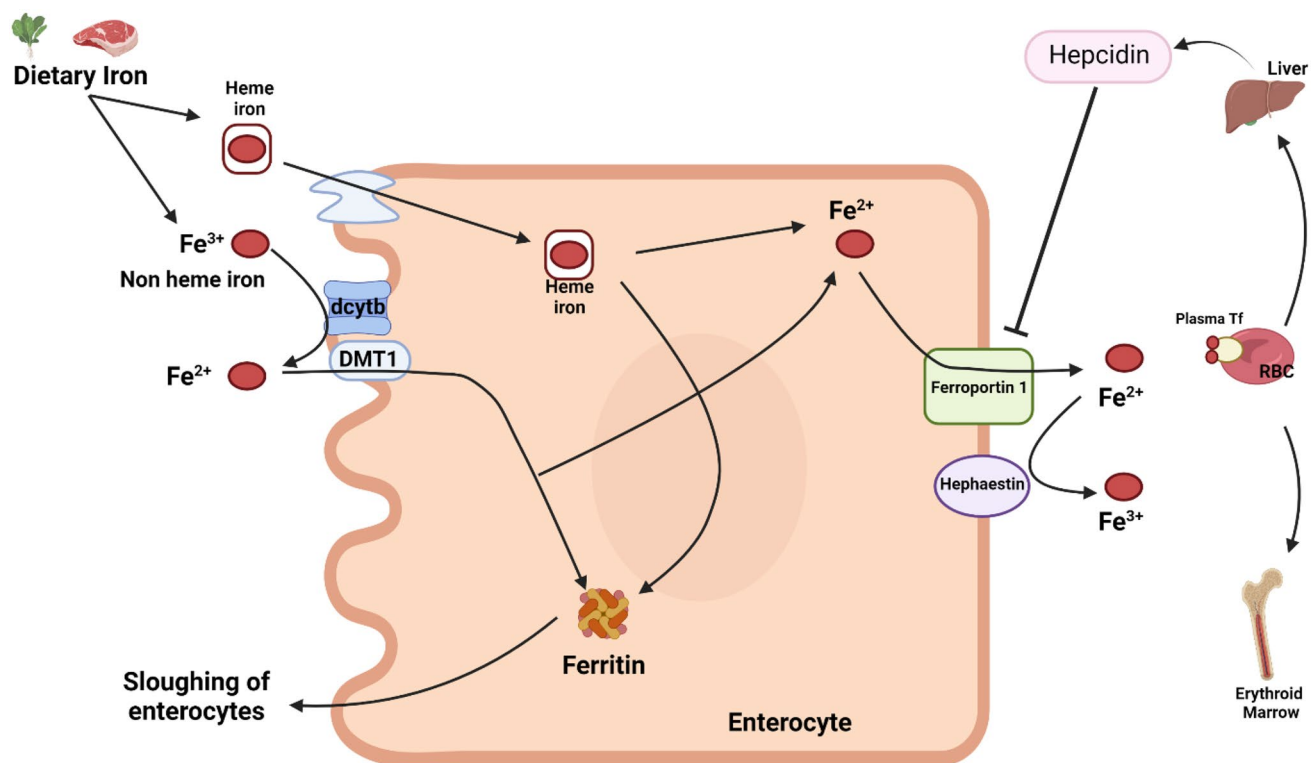
Hereditary hemochromatosis—classification, genetics, and epidemiology

Genetics of hereditary hemochromatosis

Pathogenic variants in multiple genes can result in systemic iron overload. Table 1 lists a classification of hereditary hemochromatosis modified from recommendations by the Nomenclature Committee of the International Society for the Study of Iron in Biology and Medicine [5]. Systemic iron overload can occur in several other hereditary conditions that will not be considered further here. These include β -globin disorders, such as beta-thalassemia, sideroblastic anemia, pyruvate kinase deficiency, and hereditary spherocytosis.

Forms of both *HFE*-related and non-*HFE*-related hemochromatosis result in iron overload by diminished hepcidin. Iron overload can also be the result of impairment of the export function of ferroportin.

The age of onset of iron accumulation and therefore of symptom onset differs among the different genetic causes



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Fig. 1 Intestinal Iron Absorption. Iron absorption occurs in the enterocytes of the small intestine before internalization of heme or non-heme iron. Ferrous iron is taken up by DMT1 following reduction by DCYTB. Excess iron is stored in ferritin or lost via enterocyte sloughing. Ferrous iron is exported via ferroportin, oxidized by hephaestin and transported by transferrin to various tissues for use

or storage. Hepcidin senses body iron stores and acts as a negative regulator. Hepcidin binds to the iron export protein, ferroportin, on target cells and is responsible for the internalization and degradation of ferroportin. *DMT1* divalent metal transporter 1, *dcytb* duodenal cytochrome B, *RBC* red blood cell, Fe^{3+} ferric iron, Fe^{2+} ferrous iron, *Tf* transferrin

Table 1 Modified Classification of Hemochromatosis based on Recommendations of the International Society for the Study of Iron in Biology and Medicine (modified from [5])

Novel classification	Molecular pattern	Features
<i>HFE</i> -related	p.Cys282Tyr homozygosity compound heterozygosity p.Cys282Tyr/ His63 Asp compound heterozygosity of p.Cys282Tyr with other rare pathogenic variants 106-109or <i>HFE</i> deletion.110	Variable penetrance Always consider the presence of host or environmental cofactors for iron overload and co-toxic liver injury, e.g., alcohol, metabolic-associated fatty liver disease In subjects with, p.Cys282Tyr/His63Asp compound heterozygosity or p.His63Asp homozygosity and iron overload-related disease consider referral to a specialist center to determine the need for second-line genetic testing for rarer variants May be associated with severe iron loading in multiple organs in younger populations While mutations in any hepcidin-regulatory gene may be causative, the effects of novel mutations should be confirmed through functional and epidemiological studies Molecular subtypes characterization only at specialized centers, but the diagnosis of non- <i>HFE</i> -related hemochromatosis is sufficient to start treatment at non-specialized centers
Non- <i>HFE</i> -related	Rare pathogenic variants in “non- <i>HFE</i> ” genes: <i>HJV</i> -related <i>HAMP</i> -related <i>TFR2</i> -related <i>SLC40A1</i> (GOF)-related	
Digenic	Double heterozygosity and/or double homozygosity/heterozygosity for mutations in 2 different genes involved in iron metabolism (<i>HFE</i> and/or non- <i>HFE</i>)	More commonly, p.Cys282Tyr mutation in <i>HFE</i> gene might coexist with mutation in other genes; rarely, both mutations involve non- <i>HFE</i> genes
Molecularly undefined	Molecular characterization (still) not available after sequencing of known genes (provisional diagnosis)	The case should be referred to specialized centers for further consideration

of hemochromatosis. The most common form of hereditary hemochromatosis is that due to p.Cys282Tyr homozygosity in *HFE* and manifests in adulthood. Males are more likely than females to develop iron overload and symptomatic disease. Compound heterozygous *HFE*-related hemochromatosis (p.Cys282Tyr/p.His63Asp) is usually clinically inconsequential but may cause liver injury and cirrhosis when accompanied by cofactors, such as regular moderate to high alcohol consumption, metabolic fatty liver disease, or hepatitis C infection.

However, other rarer forms of hemochromatosis are recognized and have different phenotypes. The *HFE* p.Ser65Cys substitution is not associated with excess iron storage in tissues or end organ injury and is therefore not of clinical significance. Juvenile hemochromatosis, which most commonly results from biallelic pathogenic variants in *HJV* or *HAMP*, has an onset of iron accumulation in early childhood with symptoms becoming evident as early as the first decade of life. It is often associated with severe iron overload with multiple organs involved. Males and females are affected equally by juvenile hemochromatosis. Hepcidin deficiency occurs due to pathogenic variants in the transferrin receptor 2 (*TFR2*) gene. Ferroportin disease occurs due to either reduced export function of ferroportin or to resistance of ferroportin to hepcidin.

Epidemiology

Multiple studies have examined the frequency of hemochromatosis and the underlying genetic cause in different populations. These are summarized in Table 2. By far the most common cause of hemochromatosis is homozygosity for the p.Cys282Tyr substitution in the *HFE* protein. Compound heterozygosity for p.Cys282Tyr/p.His63Asp in *HFE* is more common than p.Cys282Tyr homozygosity but has markedly lower biochemical and clinical penetrance. Among individuals diagnosed with hemochromatosis, *HFE* p.Cys282Tyr homozygosity accounts for most in Australia [6] and 96% in Brittany, France [7], while only accounting for 62% in Italy [8] and 39% in Greece [9].

The highest prevalence of *HFE* p.Cys282Tyr homozygosity is among Northern Europeans, in particular, Ireland and Scandinavia, with a lesser prevalence in Southern Europe [10]. *HFE* p.Cys282Tyr homozygosity is rare in those of African and Asian ancestry with estimates of prevalence being 1 in 6781 and 1 in 25,000, respectively [11, 12].

Hemochromatosis is a rare disorder in non-Caucasian populations. Isolated cases of C282Y homozygous hemochromatosis have occasionally been reported but the C282Y mutation has a very low frequency in the Asia Pacific region [13]. The H63D mutation is more common with a 2% allele frequency in Asian population compared with 15% in Europeans [13]. Other rare mutations in *HFE* including

Table 2 Selected population studies of the frequency of HFE p.Cys282Tyr homozygosity

Country	Study population	Cohort size	Frequency of HFE p.Cys282Tyr
Australia [23]	Workplace	11,307	1 in 221
Australia [24]	Cohort enrolled through the electoral roll (enriched for Northern European ancestry)	29,676	1 in 146
USA [11]	Primary care and blood drawing laboratories	99,711	1 in 333
United Kingdom [25]	Postal invitation to individuals registered with the National Health Service	451,243	1 in 156
Norway [26]	Hospitalized individuals (Caucasian only)	1900	1 in 136
Spain [27]	Blood donors	5370	1 in 671
France [28]	Attendees at health appraisal centers	9396	1 in 174

the E277K, Y231del and a homozygous splice mutation, IVS5 + 1 G > A have been reported [14–16]. Mutations in HJV and HAMP causing juvenile iron overload have also been reported throughout the Asia Pacific region but remain extremely uncommon. Compound heterozygous mutations of HJV or combined heterozygous mutations of the BMP/SMAD pathway genes leading to reduced hepcidin expression have been described in China [17]. Although rare, mutations in TFR-2 may be the leading cause of hemochromatosis in the Asia Pacific region where the I238M variant of TFR2 (previously reported as a polymorphism) has an allele frequency of 7% [18–20]. Ferroportin disease has a worldwide distribution with descriptions of associated iron overload in patients from the Solomon Islands, Sri Lanka, Vietnam, and India. Mutations in ferroportin that disrupt the binding of hepcidin to ferroportin and cause non-classical ferroportin disease have been described in a family from Thailand and in patients from China [13, 21, 22].

Clinical features and natural history

Clinical manifestations

The clinical manifestations of HFE p.Cys282Tyr homozygous hemochromatosis were initially appreciated by Trouseau and von Recklinghausen [29, 30]. Early clinical cohort studies described significant morbidity and mortality [31–33]. Importantly, subjects without cirrhosis were shown to have survival equivalent to control populations [31, 34]. The disorder was shown to be inherited in an autosomal recessive fashion by Simon and colleagues [33], and in tight linkage disequilibrium with the HLA complex on chromosome 6p [35], followed later by discovery of the *HFE* gene by Feder et al. [36].

Following discovery of the *HFE* gene [36], population studies demonstrated variable biochemical and clinical manifestations [11, 24, 37, 38]. Cross-sectional cohort studies suggested that hemochromatosis was not associated

with increased mortality [39–42]. More recent population studies have shown that males, but not females, homozygous for p.Cys282Tyr have a significantly increased mean risk of death by age 75 years of 19.5% compared to 15.1% for controls [43]. Compound or simple heterozygosity for p.Cys282Tyr and/or p.His63Asp was found not to be associated with increased risk of premature death [24, 25, 43, 44]. P.Cys282Tyr homozygosity was associated with excess dementia, delirium, sarcopenia, frailty, and chronic pain after the age of 60 years in males [45, 46].

Homozygosity for p.Cys282Tyr is associated with morbidity in up to 40% of males and 13% of females [11, 24, 25, 38, 47, 48]. The variable biochemical and clinical penetrance is most likely due to multiple genetic and environmental modifiers [49]. Males are likely at higher risk than females due to the absence of the protective effects of menstruation and pregnancy [50]. Symptoms are non-specific and often equally prevalent in individuals either with or without hemochromatosis [24, 37]. The commonest symptom is fatigue, which is observed mainly in males with elevated serum ferritin levels [24].

The most frequent significant clinical manifestations are liver disease and arthritis [5, 31, 34, 51, 52]. Males, but not females, who are homozygous for p.Cys282Tyr have a greater than fourfold increased risk of developing liver disease compared to those without *HFE* variants [25]. Male p.Cys282Tyr homozygotes also have increased risks of arthritis, colorectal cancer, pneumonia, and diabetes mellitus [25, 53]. P.Cys282Tyr homozygous females have a 1.3- to twofold increased risk of colorectal cancer, breast cancer, and arthritis compared to those without *HFE* gene pathogenic variants [25, 53].

Liver Disease

Advanced liver fibrosis or cirrhosis [54] in HFE-related hemochromatosis is rare under the age of 45 years in the absence of other liver co-morbidities, occurring in 8–25% of all HFE p.Cys282Tyr homozygotes [38, 51, 52, 55, 56].

Risk factors include excessive alcohol consumption, diabetes mellitus, arthritis, serum ferritin levels greater than 1000 µg/L, liver iron concentration greater than 200 µmol/g, and total mobilizable iron stores by therapeutic phlebotomy of greater than 9.6 g [47, 56–60].

HFE p.Cys282Tyr homozygous males have a 12-fold increased lifetime risk of primary liver cancer compared to those without *HFE* variants [43]. Females who are HFE p.Cys282Tyr homozygous are not at increased risk of liver cancer [43]. The greatest risk of primary liver cancer occurs in those with cirrhosis [31, 34, 61], and these individuals should be recommended to undergo routine 6-monthly liver ultrasound for liver cancer surveillance [5, 51, 52, 62]. Regression of cirrhosis to Scheuer grade F2 or less results in a reduction in the risk of liver cancer, although cirrhosis persists in the majority after treatment [61].

Arthritis

Hemochromatosis arthritis affects at least 24% of individuals and is a major cause of morbidity [63, 64]. Classically, arthropathy affects the metacarpophalangeal joints followed thereafter by hip, ankle, radiocarpal, elbow, shoulder and knee joints, as well as the lumbar spine [63, 64]. It can be challenging to discriminate between hemochromatosis arthropathy and degenerative osteoarthritis (also known as type 1 polyarticular osteoarthritis) [64]. It is unclear why arthropathy affects only a subset of people with hemochromatosis. Arthritis may occur at any point during the natural history of the disease, even following successful phlebotomy therapy [52, 64]. Risk factors for arthritis include increasing age, advanced liver fibrosis, serum ferritin levels elevated greater than 1000 µg/L, and elevated serum transferrin saturation $\geq 50\%$ for prolonged periods of time [65, 66].

Liver disease and arthritis tend to occur concomitantly. Hemochromatosis arthritis is more likely with higher iron load or more advanced liver disease [67, 68]. A recent study showed that arthritis was strongly associated with advanced liver fibrosis, and 84% of HFE p.Cys282Tyr homozygous subjects with advanced hepatic fibrosis had arthritis, while 34% p.Cys282Tyr homozygotes with arthritis had advanced hepatic fibrosis. Importantly, only 5% of subjects without arthritis had advanced hepatic fibrosis; thus, the absence of arthritis had a 95% negative predictive value for advanced liver fibrosis [57].

Other clinical manifestations

Other conditions which have been reported in HFE p.Cys282Tyr homozygous hemochromatosis include diabetes mellitus, hyperpigmentation, hypogonadotropic hypogonadism, and cardiomyopathy [25, 51, 52, 62]. These conditions are usually managed as per standard of care, and in

addition to the treatment of iron overload. Cardiomyopathy is a rare complication which is potentially reversible by iron removal therapy [69].

Secondary iron overload

Secondary iron overload is often due to a combination of increased iron absorption and recurrent blood transfusions in the setting of hemoglobinopathies, such as thalassemia and sickle cell disease. In addition, increased hepatic iron stores can be seen in chronic liver disease, such as alcoholic liver disease, chronic hepatitis C, and metabolic-associated fatty liver disease (MAFLD)[70, 71].

The role of alcohol in the development of increased liver iron has been clarified in recent years. Alcohol can result in increased intestinal iron absorption due, in part, to alcohol-induced down-regulation of hepcidin expression and altered intestinal iron transporters [72]. In alcoholic subjects, Perls' method of staining liver sections often reveals stainable iron in Kupffer cells probably reflecting iron release from damaged hepatocytes. Hemochromatosis subjects who consume greater than 60 g of alcohol per day are nine times more likely to develop cirrhosis than those who drink less than this amount [73].

Mild increases in liver iron stores can be seen in patients with manifestations of the metabolic syndrome. Such patients also have elevated serum ferritin concentration with normal or moderately increased transferrin saturation. This condition is known as the dysmetabolic iron overload syndrome [74]. It is worth noting that many subjects with MAFLD have elevated serum ferritin concentration with normal iron stores. Quantitative assessment of tissue iron by MRI scan is useful in these circumstances. There is no conclusive evidence to support the role of phlebotomy in patients with mild increases in liver iron stores or MAFLD. However, it is worth noting that hepatic stellate activation was detected in liver biopsies of hemochromatosis patients with a hepatic iron concentration of 60 µmol/g dry weight [75], suggesting that this modest degree of iron loading may be important in the setting of other hepatic co-toxins.

Iron toxicity and hepatic fibrogenesis

Iron toxicity

Abnormal iron homeostasis in HFE-hemochromatosis can lead to a wide variety of different iron-induced pathologies, including arthritis, cardiomyopathy, diabetes, cirrhosis, and HCC. Excessive iron deposition in the liver leads to the generation of reactive oxygen species (ROS), oxidative damage to intracellular organelle membranes and DNA strand

breaks, and subsequent organelle dysfunction and liver cell injury [62, 76, 77], that can lead to cell death via apoptosis, necrosis, or iron-dependent ferroptosis [78, 79]. Both free iron and labile iron, including non-transferrin-bound iron, catalyze the formation of ROS via the Fenton and Haber–Weiss reactions, which can impact hepatic mitochondrial, microsomal, and lysosomal function [80–82]. Therefore, homeostatic regulation of intracellular iron via binding to ferritin or hemosiderin under normal physiological conditions is critical. Under conditions of excessive iron overload as seen in HFE-hemochromatosis, normal mechanisms of antioxidant defense that include glutathione peroxidase, catalase, and superoxide dismutase [83] are overwhelmed.

Hepatic fibrogenesis

Iron-induced liver cell injury results in the release of cell damage-associated cytokines, chemokines, and proinflammatory mediators, which leads to hepatic inflammation [84], the activation of hepatic stellate cells into pathological collagen-producing myofibroblasts, and ultimately hepatic fibrosis, cirrhosis, and liver cancer [77]. In recent years there has been conjecture over the role of iron per se in liver pathology as iron toxicity studies have largely been conducted on in vitro cultured liver cells with relatively scant in vivo evidence either in animal models of iron overload or in human HFE-hemochromatosis liver tissue [85]. Indeed, a recent proposal suggests that in conditions where iron is proposed to be pathogenic, iron-induced liver damage may in fact be potentiated by coexistent inflammation, with subsequent necrosis and hepatic stellate cell activation driving fibrosis [85]. While iron and transferrin have been shown to induce hepatic stellate cell activation and collagen expression in vitro [86–88], in vivo models of iron overload demonstrate only minor fibrosis, although the numbers of alpha-smooth muscle actin (α SMA)-positive myofibroblasts were significantly increased [89, 90]. In humans with HFE-hemochromatosis, studies have shown a significant positive correlation between the numbers of α -SMA-positive hepatic stellate cells and increasing hepatic iron concentration, suggesting a causal link [75]. However, that study demonstrated that in early disease these cells are located in zone 3 of the hepatic acinus, distal to the zone 1 iron-laden hepatocytes, implying hepatic stellate cells are activated into collagen-producing myofibroblasts by soluble mediators released from iron-damaged hepatocytes rather than by iron per se [75, 85]. Regardless of the mechanisms responsible for liver pathology, hepatic fibrosis is reversible with iron removal via phlebotomy therapy [47], with the risk of developing liver cancer significantly diminished if fibrosis staging is reduced to F2 or less [61]. Human studies have shown that as the severity of liver fibrosis progresses, the number of hepatic progenitor cells increase, mimicking the pathological processes associated with the “wound healing” and carcinogenic response of

the liver to injury which is observed in chronic viral hepatitis and alcohol-related liver disease [91, 92].

While HFE-hemochromatosis has traditionally been viewed as a non-inflammatory condition, there is growing evidence that suggests the disease does indeed encompass an inflammatory component. Tissue ferritin is released by iron-damaged hepatocytes and Kupffer cells in iron overload conditions and has been shown to act as an iron-independent proinflammatory mediator of hepatic stellate activation as a portent to fibrogenesis [84], acting via a high affinity binding protein for H-subunit ferritin (FTH) [93]. Serum ferritin levels have been demonstrated to be a better predictor of fibrosis severity than hepatic iron concentration, independent of gender, steatosis, or alcohol consumption [59], adding weight to the observations linking ferritin to hepatic stellate cell activation. Iron loading of hepatocytes in patients with HFE-hemochromatosis has been shown to lead to impaired hepatocyte replication and senescence, which was correlated with serum ferritin levels, hepatic iron concentration and oxidative stress, which stimulates the ductular reaction and fibrosis [91]. In that study, portal inflammation was shown to occur in HFE-hemochromatosis and was independently associated with the ductular reaction and fibrosis [91], demonstrating the potential mechanism of iron overload-induced injury and fibrogenesis in this condition. In HFE-hemochromatosis, it is the accumulation of excess Kupffer cell iron, which follows progressive and sustained accumulation of excessive iron within hepatocytes, that appears to be a necessary precursor or trigger for the development of hepatic fibrogenesis [94]. This being said, many patients with HFE-hemochromatosis do not develop severe fibrosis even in the presence of significant iron overload, which suggests that in addition to iron and inflammation, there remains a role for environmental modifiers, such as fat, excessive alcohol consumption and diabetes mellitus, as well as genetic modifiers, of the fibrogenic processes associated with this disease [49, 60, 95, 96], including gene variants of *PNPLA3* and *PCSK7* which have been shown to associate with increased liver disease risk [97–99].

It is in the context of the current knowledge outlined above that these clinical practice guidelines for the management of hemochromatosis have been developed to assist clinicians in the day-to-day care of affected patients and their relatives.

Question 1: is general population screening for hemochromatosis indicated? If not, and in the absence of a relevant family history, then who should be tested for hemochromatosis?

General population screening for hemochromatosis has not been recommended due to variable and incomplete penetrance and a lack of any proof of resulting survival

advantage [51, 52, 100, 101]. However, the recent report of significantly increased mortality in adult male p.Cys282Tyr homozygotes compared with those without HFE variants in the UK Biobank study supports re-examination of the utility of screening in susceptible male populations [43]. Screening is indicated in first-degree relatives of probands and is discussed in more detail later [51, 52].

Patients with symptoms, signs, or biochemical abnormalities that could be consistent with hemochromatosis should undergo measurement of serum iron indices. Fatigue is common and may be the only presenting symptom. Hemochromatosis should be considered in type 2 diabetic patients, and patients with loss of libido, unexplained cardiac failure or cardiac arrhythmias and significant polyarthropathy—particularly if the 2nd and 3rd metacarpophalangeal joints are involved. Abnormal liver function tests should prompt consideration of hemochromatosis, even if there is another diagnosis because MAFLD and alcoholic liver injury are common in patients with underlying hemochromatosis. On occasions, biochemical evidence of iron overload can be seen unexpectedly—for example, when considering iron deficiency as a cause of fatigue in menstruating females. These patients should be managed in the same manner as those with symptoms of iron overload.

Recommendation:

General population screening is not recommended but individuals of European descent with any clinical symptoms or signs compatible with the diagnosis or a family history of iron overload should be evaluated with genetic testing and measurement of serum transferrin saturation and ferritin levels. Patients with symptoms, signs, or biochemical abnormalities consistent with hemochromatosis should undergo measurement of serum ferritin concentration and transferrin saturation (HIGH QUALITY of EVIDENCE; STRONG RECOMMENDATION) (Fig. 2).

Question 2: should screening be offered to first-degree relatives of patients with hemochromatosis? If so, what tests should be used and what age should screening for hemochromatosis take place? How frequently should hemochromatosis patients with normal serum ferritin at diagnosis be retested?

Because the carrier frequency of each of the two common HFE pathogenic variants, p.Cys282Tyr and p.His63Asp, is very high among people of European ancestry, screening should be offered to the first-degree relatives of individuals who have HFE-related hemochromatosis. In relation to

children of affected individuals, options include offering testing to each child or testing the reproductive partner of the affected individual. If the reproductive partner has neither the p.Cys282Tyr nor the p.His63Asp variant, then their offspring do not need to have further testing as their risk of HFE-related hemochromatosis is low [102]. This latter approach is recommended where the couple have more than one child as it is likely that fewer family members will require testing.

We recommend that testing be offered from late adolescence onwards. Data from the ironXS high school screening program showed that individuals identified with HFE p.Cys282Tyr homozygosity in late adolescence are very unlikely to have dangerously high body iron levels as measured by serum ferritin [103]. By offering this testing in late adolescence, the at-risk individual can make an informed decision as to whether or not they wish to have this testing.

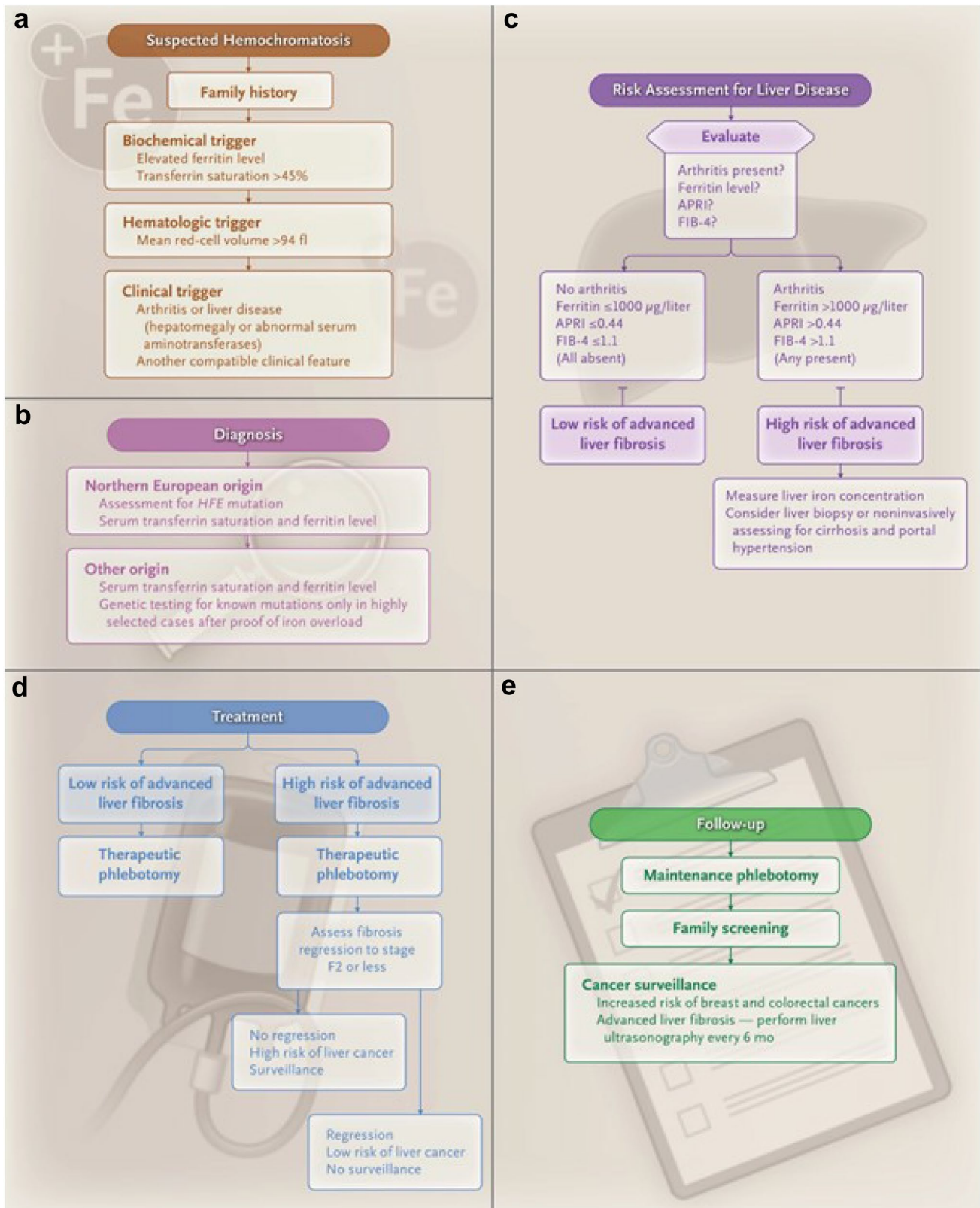
Recommendation

We recommend that individuals found to be homozygous for the HFE p.Cys282Tyr pathogenic variant or compound heterozygous for p.Cys282Tyr/p.His63Asp with normal serum ferritin, should have measurements of serum ferritin and transferrin saturation repeated no more frequently than once a year and up to once every 5 years, especially if previously stable and not rising [51, 95]. While it is unlikely that serum ferritin would increase from the normal range to a dangerously high level in 12 months, clinical experience suggests that recommending testing beyond annually presents a higher risk of people forgetting to undertake this test.

Despite the observation of significant concordance of disease expression between siblings with hemochromatosis, we also recommend testing of first-degree relatives of subjects without phenotypic expression for HFE p.Cys282Tyr and p.His63Asp variants. Parents and siblings of an affected individual should be tested for the HFE p.Cys282Tyr and p.His63Asp variants (LOW QUALITY of EVIDENCE; STRONG RECOMMENDATION).

Question 3: what biochemical iron parameters are best used to test patients for hemochromatosis?

While generalized population screening for iron overload is not recommended [104], patients with suspected iron overload are identified by elevated serum ferritin and transferrin saturation > 45% [52, 105, 106] followed by genetic testing for HFE p.Cys282Tyr and p.His63Asp. Screening using both serum ferritin and transferrin saturation will identify the majority of individuals who will go on to develop iron overload [107]. While widely used, both transferrin saturation



◀**Fig. 2** Recommended Key Steps in the Clinical Evaluation of Hemochromatosis. Features which raise the need to suspect hemochromatosis are shown in Panel A (it is possible that more than one of these may be present at any one time), how to diagnose hemochromatosis in Panel B (the arrows represent the choice of options to consider), and how to assess for liver disease in Panel C. Panel D shows treatment, and Panel E shows follow-up (the arrows represent the range of issues to consider). APRI denotes aspartate aminotransferase-to-platelet ratio index, and FIB-4 Fibrosis-4 index. From *The New England Journal of Medicine*, John K. Olynyk and Grant A. Ramm, Hemochromatosis, Volume 387:2159–70. Copyright © (2022) Massachusetts Medical Society. Reprinted with permission, <https://www.nejm.org/doi/full/10.1056/NEJMra2119758> [95]

and serum ferritin have limitations. Transferrin saturation has high biological variability and low sensitivity to detect HFE-related hemochromatosis [108] and a retrospective review of outpatient referrals for elevated serum ferritin found 64% of patients with a serum ferritin threshold over 1000 µg/L did not have iron overload on biopsy [109]. Age and gender should be considered when assessing elevation of serum ferritin. Serum ferritin increases steadily in males until the sixth decade of life, while serum ferritin is lower in females of all ages but increases sharply post menopause [110]. Other biochemical triggers that may lead one to suspect hemochromatosis include mean red cell volume > 94 fl, and altered liver enzymes and clinical triggers include hepatomegaly and arthritis (Fig. 2A). The sensitivity and specificity for MCV > 94 fL are 67% and 91% for females, and 50% and 89% for males.

Recommendation

The best initial tests are serum ferritin and transferrin saturation followed by genetic testing if both are elevated. Liver biopsy, non-invasive measures of liver fibrosis (see Question 7) or MRI can then be performed to assess level of hepatic iron loading (see Question 6) and liver fibrosis (HIGH QUALITY of EVIDENCE; STRONG RECOMMENDATION) (Fig. 2).

Question 4: who should undergo genetic testing for the p.Cys282Tyr/p.His63Asp variants in HFE?

Genetic testing for HFE p.Cys282Tyr and p.His63Asp should be offered to all individuals of European descent found to have both raised serum ferritin and transferrin saturation, individuals with an isolated raised serum ferritin where no other cause is apparent [111], as well as first-degree relatives of individuals with HFE-related hemochromatosis (see Question 2). There are no clear values of

serum ferritin and transferrin saturation that should trigger such genetic testing [106]. The upper end of the accepted reference range for serum ferritin levels in Australia is 620 µg/L in males. 220 µg/L and 370 µg/L are the upper end of reference range for premenopausal and postmenopausal females, respectively [112, 113]. A transferrin saturation over 45% in both males and females is considered elevated [114]. If biallelic HFE pathogenic variants are not identified, then testing of other genes associated with iron overload should be considered (see section on Genetics of Hemochromatosis and Question 5). A person with severe iron overload who is found to be compound heterozygous for HFE p.Cys282Tyr/p.His63Asp should be offered testing of other genes known to cause severe iron overload since this genotype generally results in either no iron overload or mild iron overload.

Recommendation

Genetic testing for HFE p.Cys282Tyr and p.His63Asp should be offered to all individuals particularly those of European descent having both raised serum ferritin and transferrin saturation, individuals with an isolated elevated serum ferritin or transferrin saturation where there is no other cause identified, and first-degree relatives of individuals with HFE-related hemochromatosis (HIGH QUALITY of EVIDENCE; STRONG RECOMMENDATION) (Fig. 2).

Question 5: who should be tested for non-HFE variants?

Patients with proven iron overload without HFE variants are occasionally encountered in clinical practice. It is essential to confirm the presence of hemochromatosis before pursuing non-HFE variants as abnormal serum iron indices are often due to other liver diseases, such as MAFLD [111]. It is also important to exclude other causes of secondary iron overload, including thalassemia or parenteral iron administration. It is advised that patients with confirmed — but unexplained — iron overload should be referred to a specialist in iron disorders for further assessment. Genetic testing for non-HFE variants is available in specialized centers (*HAMP, HJV, TFR2, TF, CP, BMP6, SCL40A1*) and discussions with personnel experienced in this field is important to evaluate the likelihood that any identified variants of genes of interest are pathogenic [13]. First-degree relatives of patients with non-HFE mutations should undergo testing.

Recommendation

Patients with unexplained proven iron overload, particularly those of non-European descent, should be evaluated by a specialist in iron overload disorders, and results of genetic testing should be discussed with those experienced in the field to advise on pathogenicity of identified variants (MODERATE QUALITY of EVIDENCE; STRONG RECOMMENDATION).

Question 6: how is liver iron concentration quantified in a suspected case of hemochromatosis?

Quantitation of liver iron concentration is important in the assessment of the risk of advanced liver fibrosis and primary liver cancer and should routinely be performed in all individuals with serum ferritin levels $> 1000 \mu\text{g/L}$, arthritis, or otherwise judged to be at risk due to clinical or biochemical features of liver disease.

Quantitation of liver iron concentration has historically been undertaken invasively via liver biopsy, although more recently reliable methods have been described for non-invasive measurement [5, 51, 52, 58, 115]. Practical non-invasive methods include retrospective calculation of the iron removed based on the number and volume of therapeutic phlebotomies undertaken to reduce the serum ferritin level to 50–100 $\mu\text{g/L}$, based on a 500 mL phlebotomy removing approximately 250 mg of iron [51, 94], as well as magnetic resonance imaging. A number of differing magnetic resonance methods of liver iron deposition and their correlations with liver biopsy biochemical measurements have been described [116–120]. Allowing for the heterogeneity of iron deposition [119, 121, 122], magnetic resonance imaging provides good clinical utility for quantification of iron overload [52, 116, 119]. Magnetic resonance imaging is also accurate for quantification of myocardial iron deposition [123]. There are numerous different MRI methods for quantification of liver iron concentration [124, 125] some of which can be confounded by the presence of steatosis, fibrosis, or inflammation. The FDA-approved spin-density projection-assisted R2-MRI method of liver iron measurement (FerriScan®) has been shown to give results unconfounded by the presence of steatosis, stage of fibrosis, and grade of necroinflammation [119, 126, 127]. Of the published magnetic resonance methods for measuring liver iron concentration, only that described by St Pierre et al. [119] has been approved for human use by regulatory authorities in the United States of America, Europe, and Australia.

Recommendation

All subjects with hemochromatosis and serum ferritin levels elevated to $> 1000 \mu\text{g/L}$ should undergo measurement of hepatic iron concentration using available validated methods, including MRI, as described above and chemical estimation [128] (HIGH QUALITY of EVIDENCE; STRONG RECOMMENDATION).

Question 7: how is hepatic fibrosis assessed in hemochromatosis?

The staging of hepatic fibrosis at diagnosis is necessary to assess for severity of liver injury and end organ damage in order to better guide clinical management, and in those with advanced fibrosis or cirrhosis, to screen for primary liver cancer. Assessment of progressive fibrosis is clearly warranted, as is regression of advanced fibrosis with phlebotomy therapy [47], which can guide the requirement for ongoing HCC surveillance. Recent evidence shows regression of biopsy-proven advanced fibrosis and cirrhosis with iron removal [61, 129], where 70% of patients with F3 and 20% of patients with F4 fibrosis showed regression of fibrosis stage over a median follow-up of 9.5 years [61]. The amount of iron removed via phlebotomy, or ‘mobilizable iron,’ to achieve iron depletion (reflected in a resultant serum ferritin $< 100 \mu\text{g/L}$) has been shown to correlate with the severity of fibrosis, with a recent study demonstrating that advanced fibrosis can be predicted above a threshold of 9.6 g of iron removed, with an area under the receiver operating characteristic (AUROC) curve of 0.92 or an hepatic iron concentration above 200 $\mu\text{mol/g}$ (AUROC 0.83) [47, 56, 130]. Thus, understanding the potential burden of mobilizable body iron stores via phlebotomy may permit better stratification of patients requiring more sophisticated and costly assessment of liver disease complications [56].

In HFE-hemochromatosis, liver disease and arthritis can occur concurrently [95], with arthritis more likely in patients with higher body iron load and/or advanced hepatic fibrosis [67, 68]. Arthritis was recently shown to be strongly associated with the presence of advanced fibrosis, with 84% of patients with advanced fibrosis also having arthritis [57]. Of interest, the absence of arthritis had a 95% negative predictive value for advanced fibrosis [57].

Liver biopsy remains the ‘gold standard’ to assess for hepatic pathology in HFE-related hemochromatosis [51], although it is now used infrequently. If cirrhosis is suggested via clinical examination or ultrasound,

a liver biopsy may not be recommended [106]. Elevated serum ferritin levels $> 1000 \mu\text{g/L}$ can assist in identifying 20–45% of patients who would benefit from a liver biopsy [58, 131, 132], with several studies showing $< 2\%$ of HFE-hemochromatosis patients with ferritin $< 1000 \mu\text{g/L}$ at diagnosis have bridging fibrosis or cirrhosis in the absence of other co-morbidities, with a 94% negative predictive value [58, 131]. The addition of elevated aspartate aminotransferase and a platelet count $< 200 \times 10^9/\text{L}$ to a serum ferritin $> 1000 \mu\text{g/L}$ has been reported to predict cirrhosis in 80% of patients [58]. However, in another study, application of this algorithm did not detect 30% of cirrhotic patients [133]. Rather, that study demonstrated that elevated serum hyaluronic acid $> 46.5 \text{ ng/mL}$ predicted 100% of patients with cirrhosis and when using a serum ferritin $> 1000 \mu\text{g/L}$ to triage those for biopsy, obviating the need for a liver biopsy in 60% of patients with HFE-hemochromatosis [133]. Measurement of hyaluronic acid is not used in routine clinical practice limiting its potential utility, and others have reported its relative lack of diagnostic accuracy, albeit in a study limited only to patients with a serum ferritin $> 1000 \mu\text{g/L}$ [134].

Far fewer liver biopsies are performed in the modern era with the advent of non-invasive modalities to quantify hepatic iron [119, 135, 136], and to detect and stage liver disease, thus prospective studies to assess the utility of contemporary non-invasive technologies matched against liver biopsy-staged fibrosis in HFE-hemochromatosis are increasingly unlikely. Many newer non-invasive methods to detect and stage liver disease, such as direct and indirect serum fibrosis biomarker panels and elastography, have been validated in chronic viral hepatitis or MAFLD (reviewed in [137]), and a few such studies are also emerging in cohorts of patients with HFE-hemochromatosis.

A study by Legros and colleagues demonstrated the potential utility of transient elastography to detect advanced fibrosis in patients with HFE-hemochromatosis [134]. In patients with a serum ferritin $> 1000 \mu\text{g/L}$, a liver stiffness measurement $< 6.4 \text{ kPa}$ was proposed to exclude advanced fibrosis, whereas a value $> 13.9 \text{ kPa}$ was predictive of advanced fibrosis [134]. While transient elastography is recommended in recent clinical guidelines [106], caution should be exercised as that study only included patients with raised transaminases or a serum ferritin $> 1000 \mu\text{g/L}$, with only 15 patients having advanced fibrosis and the proposed elastography cut-offs only correctly diagnosing 61% of patients in the study. Of interest using these cut-off values restricted the requirement of liver biopsy to 39% of patients with indeterminate or invalid liver stiffness measurements, which was a similar observation to a previous study using a

combination of elevated serum ferritin and serum hyaluronic acid [133].

Hepascore (which uses an algorithm based on age and gender, and serum levels of γ -glutamyl transpeptidase (GGT), hyaluronic acid, bilirubin, and α 2-macroglobulin [138, 139]) and transient elastography were assessed in a study of HFE-hemochromatosis patients without matched liver biopsy-staged fibrosis [140]. This study used cut-offs for advanced fibrosis based on biochemical panels (as discussed below) and showed that advanced fibrosis could be detected but only in those with a serum ferritin $> 1000 \mu\text{g/L}$. Once again caution in interpretation is required as this study was not matched to liver biopsy-validated fibrosis staging.

As mentioned, a recent study has evaluated the utility of simple, inexpensive biochemical panels that can be determined via routine liver function tests in patients with HFE-hemochromatosis [141]. In that study, serum from 181 patients with HFE-hemochromatosis who had undergone liver biopsy for their clinical management were assessed for aspartate aminotransferase-to-platelet ratio index (APRI), fibrosis-4 (FIB-4), and gamma-glutamyl transferase (GGT)-platelet ratio (GPR). An APRI score > 0.44 and a FIB-4 score > 1.1 were both demonstrated to detect liver biopsy-validated advanced fibrosis with 81% diagnostic accuracy [141]. These data indicate that thresholds for the diagnosis of advanced fibrosis in HFE-hemochromatosis are lower than those observed for more overtly inflammatory chronic liver diseases, such as chronic viral hepatitis, alcoholic liver disease, and MAFLD [106]. That study recommended that patients who do not meet the threshold for advanced fibrosis should proceed to therapeutic phlebotomy, while in those patients who breached these thresholds, a liver biopsy should be performed to confirm advanced fibrosis, and if present, these patients should undergo routine surveillance for complications, such as oesophageal varices and HCC [141]. The study also followed 64 patients after de-ironing via phlebotomy and demonstrated a significant decrease in APRI, FIB-4, and GPR in patients across the spectrum of fibrosis staging, and in a subset with available post-treatment liver biopsies, decreased APRI and GPR scores reflected fibrosis regression [141]. Finally, that study showed that assessing APRI post-phlebotomy predicted that 87% of patients with advanced fibrosis at diagnosis decreased to APRI levels indicative of mild F1–F2 fibrosis [141], suggesting that routine assessment of APRI may be clinically useful for monitoring the regression of fibrosis with treatment. These data require validation by others but such non-invasive biomarker panels show promise at least as a screening tool to determine the requirement for subsequent liver biopsy assessment of advanced fibrosis [95].

Recommendation

Liver biopsy should be considered in patients with verified HFE-hemochromatosis to assess for advanced hepatic fibrosis if any of the following non-invasive markers are exceeded:

Serum ferritin > 1000 µg/L (HIGH QUALITY of EVIDENCE; STRONG RECOMMENDATION); hepatic iron concentration > 200 µmol/g by MR methods (MODERATE QUALITY of EVIDENCE; STRONG RECOMMENDATION); APRI > 0.44; FIB-4 > 1.1; mobilizable iron stores > 9.6 g (MODERATE QUALITY of EVIDENCE; STRONG RECOMMENDATION); and transient elastography liver stiffness measurement > 13.9 kPa (HIGH QUALITY OF EVIDENCE; STRONG RECOMMENDATION) (Fig. 2).

Transient elastography < 6.4 kPa predictive of the absence of advanced hepatic fibrosis (LOW QUALITY of EVIDENCE; WEAK RECOMMENDATION), a serum ferritin < 1000 µg/L (HIGH QUALITY of EVIDENCE; STRONG RECOMMENDATION), and the absence of arthritis (MODERATE QUALITY of EVIDENCE; STRONG RECOMMENDATION) are non-invasive assessments most predictive for the absence of advanced hepatic fibrosis in HFE-hemochromatosis.

Question 8. how should patients with HFE p.Cys282Tyr/p.His63Asp compound heterozygosity or p.His63Asp homozygosity be managed?

It is very uncommon for patients with p.Cys282Tyr/p.His63Asp compound heterozygosity to develop severe iron loading and studies have also shown that iron overload is rare in individuals homozygous for p.His63Asp [142]. As such, it is important to accurately evaluate the phenotype of such subjects and identify factors that may alter serum iron indices and/or increase hepatic iron stores. One can consider testing of genes other than HFE in patients with these variants and severe iron overload—although that should occur in specialized centers.

The management of these individuals is determined by their phenotype and phlebotomy commenced if iron overload is proven. Risk factors for liver disease and secondary iron overload should be addressed, including attention to alcohol consumption and obesity [73, 143]. Patients who are identified as p.Cys282Tyr/p.His63Asp compound heterozygotes but without iron overload can undergo routine monitoring of their iron indices although in practice their chance of developing significant iron overload is low. It is unclear if these subjects with mild iron overload benefit from phlebotomy—but it is likely that they will have the same

gain in quality of life as p.Cys282Tyr homozygotes with mild iron overload [144].

Recommendation

It is important to accurately evaluate the phenotype of p.Cys282Tyr/p.His63Asp compound heterozygous subjects and identify factors that may alter serum iron indices and/or increase hepatic iron stores or cofactors for liver disease expressivity.

Annual monitoring with measurements of serum ferritin and transferrin saturation of subjects with p.Cys282Tyr/p.His63Asp compound heterozygosity or p.His63Asp homozygosity and normal iron indices is acceptable recognizing the probability of developing significant iron overload is low. Subjects with p.Cys282Tyr/p.His63Asp compound heterozygosity or p.His63Asp homozygosity and mild iron overload are unlikely to develop significant iron overload but can be managed with phlebotomy if symptomatic. Annual monitoring of iron studies in these subjects is recommended if phlebotomy is not commenced. Patients with p.Cys282Tyr/p.His63Asp compound heterozygosity or p.His63Asp homozygosity with significant iron loading should be managed with phlebotomy and referred to a specialized center for possible testing for mutations in related genes (HIGH QUALITY of EVIDENCE; STRONG RECOMMENDATION).

Question 9: at what level of serum ferritin should therapy with phlebotomy commence? What is the goal of phlebotomy treatment and how frequently should biochemical indices be monitored?

Whilst most laboratories report that serum ferritin concentration is elevated if the value is > 300 µg/L in males and 200 µg/L in females, it is well recognised that serum ferritin concentration varies with age as well as gender [110]. Therefore, consideration of these factors, the transferrin saturation, and the variable phenotype are key to the interpretation of iron studies, and by inference, the need for phlebotomy in patients with hemochromatosis.

Despite some minor variations, there is general consensus between international guidelines on the core aspects of management of patients with hemochromatosis [52, 106]. All guidelines agree that excess iron should be removed by phlebotomy, and treatment should be commenced early with an initial de-ironing phase followed by a maintenance phase to keep serum ferritin concentration in the low-normal range. Phlebotomy does not need to commence in adult p.Cys282Tyr/p.His63Asp homozygous patients, with a serum ferritin < 300 µg/L in male patients and 200 µg/L

in female patients—as many of these patients will not subsequently develop significant iron overload. Monitoring of iron studies and liver function tests on an annual basis is appropriate.

Phlebotomy is recommended in patients with elevated serum ferritin concentration. Studies have shown that patients who have a serum ferritin concentration of $> 1000 \mu\text{g/L}$ are at a high risk of hepatic cirrhosis and require investigation to determine the extent of hepatic fibrosis, as well as commence venesection therapy [108, 131, 133]. Patients with serum ferritin concentration above the reference range but less than $1000 \mu\text{g/L}$ are at risk of progressive iron accumulation and subsequent target organ damage and should undergo phlebotomy. A blinded study comparing reduction of body iron stores versus sham treatment demonstrated benefit for those with such iron indices [144].

The goal of phlebotomy is to reach a target serum ferritin of $50 \mu\text{g/L}$ in the induction (de-ironing) phase and maintain a serum ferritin concentration of $50\text{--}100 \mu\text{g/L}$ during the maintenance phase. In the initial de-ironing phase, 500 mL of blood can be removed on a weekly basis if tolerated by the patient. On occasions the amount can be increased to 1000 mL or reduced to 250 mL depending on the patient's tolerance, and likewise the frequency can be reduced to fortnightly. The frequency of phlebotomy in the maintenance phase varies but is generally required every 2–4 months. The target serum ferritin concentration can be relaxed somewhat if the patient is finding difficulties tolerating such a low serum ferritin concentration. Despite being an effective and safe therapy, some patients find difficulty complying with venesection due to a variety of causes, including lack of motivation, needle phobia, difficult venous access, and concomitant iron loading anemias [145]. Careful alterations to venesection protocols or addition of chelation therapy can be considered.

It is important to monitor hemoglobin concentration and serum ferritin during both the induction phase and maintenance phase. Measurement of hemoglobin at each phlebotomy is recommended, whereas serum ferritin can be measured at every fourth phlebotomy during the early induction phase but increased to every phlebotomy when the serum ferritin reaches $200 \mu\text{g/L}$. Serum ferritin should be monitored 2–3 times per year during the maintenance phase and the frequency of phlebotomy adjusted accordingly. Non-compliance with venesection is more common in the maintenance phase and such patients are at risk of reaccumulating iron and associated complications [145].

Unexplained reductions in the need for phlebotomy should be investigated as occult blood loss may be an underlying cause. On occasions, no explanation for the reduced need can be identified, suggesting that there may

be some intra-individual variation in phenotypic expression [146].

Recommendation

Patients with elevated serum ferritin concentration should commence a venesection program. The generally accepted target range for ferritin is $50 \mu\text{g/L}$ in the de-ironing phase and $50\text{--}100 \mu\text{g/L}$ in the maintenance phase. During the induction phase, measurement of hemoglobin at every venesection and serum ferritin at every fourth venesection is recommended until the serum ferritin concentration reaches $200 \mu\text{g/L}$ after which serum ferritin should be measured at each venesection. During the maintenance phase, measurements of serum ferritin are recommended 2–3 times per year and venesection schedule adjusted accordingly (HIGH QUALITY of EVIDENCE; STRONG RECOMMENDATION) (Fig. 2).

Question 10: when should erythrocytapheresis be considered?

Erythrocytapheresis is a method of red blood cell removal where whole blood is drawn from the patient, centrifuged to separate whole blood into plasma and red cells and then plasma is returned to the individual. Therefore, important blood components, including plasma proteins, clotting factors, and platelets, are returned to the individual being treated. Studies have shown that erythrocytapheresis can normalize serum ferritin in a shorter timeframe with fewer procedures than phlebotomies and is less likely to result in symptoms of hypovolemia [147–149]. This procedure can remove up to four times as many red cells per treatment than phlebotomy [150]. Disadvantages include that erythrocytapheresis requires specialized equipment and expert staffing that is not universally available and, that where it is available, it may be considerably more distant from a person's residence than a venue where phlebotomy can be performed. In addition, erythrocytapheresis is more expensive than phlebotomy.

Recommendation

Erythrocytapheresis should be considered for individuals who have problems with symptoms from hypovolemia from phlebotomy, those with cardiac morbidity, hypoproteinemia, and/or thrombocytopenia [150] (MODERATE QUALITY of EVIDENCE; STRONG RECOMMENDATION).

Question 11: what is the role of iron chelation in hemochromatosis?

Iron chelation is an alternative to phlebotomy therapy for those who cannot tolerate the procedure for medical or personal reasons. Chelation therapy may take the form of oral or parenteral approaches. Of these approaches, oral deferasirox is probably the best tolerated of the options [151]. Alternatively, erythrocytapheresis may be undertaken (see Question 10 above).

Recommendation

Iron chelation is an alternative to phlebotomy/erythrocytapheresis therapy for those who cannot tolerate the procedure for medical or personal reasons (LOW QUALITY of EVIDENCE; STRONG RECOMMENDATION).

Question 12: how is juvenile hemochromatosis best managed?

Juvenile hemochromatosis is an autosomal recessive disorder which manifests usually under the age of 30 years with significant iron overload. It is rare and falls under the classification of non-HFE-hemochromatosis. It can be caused by homozygous mutations in *HJV* or *HAMP*, which both result in loss of production of hepcidin and subsequent iron overload [152, 153]. It is best managed by early diagnosis and phlebotomy therapy to reduce body iron stores [5].

Recommendation

Individuals presenting with iron overload less than 30 years of age should undergo genetic testing for *HFE*- and non-*HFE*-hemochromatosis. Accurate assessment of the degree of iron overload affecting the liver and heart followed by early phlebotomy treatment offers the best prognosis (MODERATE QUALITY of EVIDENCE; STRONG RECOMMENDATION).

Question 13: what dietary modifications are recommended in hemochromatosis?

Many patients with hemochromatosis have hepatic co-morbidities, including MAFLD and alcoholic liver disease—both of which can accelerate progression of the underlying liver disease [73, 143]. In this context, it is recommended that affected patients adopt healthy lifestyles following appropriate recommendations around dietary intake and alcohol ingestion aiming to maintain a normal body mass index [114]. Dietary iron intake, for example, by ingesting red meat, does not need to be restricted, but iron-containing supplements should be avoided. Black tea and non-citrus fruit may possibly

reduce iron accumulation [154, 155]. It has been suggested that vitamin C can increase iron absorption and worsen the hemochromatosis phenotype. Limiting the intake of vitamin C supplementation to the recommended daily intake would seem appropriate [51, 156]. *Vibrio vulnificus* is a pathogen that can contaminate seafood (e.g., oysters) and hemochromatosis patients with high levels of circulating iron may be susceptible to life-threatening infections following exposure [157, 158].

Recommendation

Patients with hemochromatosis should adopt a healthy lifestyle, including maintaining normal body weight and limiting alcohol consumption [114]. Dietary iron intake does not need to be restricted, but iron supplements should be avoided (MODERATE QUALITY of EVIDENCE; STRONG RECOMMENDATION).

Question 14: when should patients with hemochromatosis undergo surveillance for HCC?

Patients with cirrhosis due to underlying hemochromatosis have a 100-fold increased risk of developing HCC. Surveillance using six-monthly ultrasounds has been shown to lead to earlier diagnosis and improved survival in patients with cirrhosis from other causes of liver disease [159–162]. It is likely that these benefits extend to patients with hemochromatosis and cirrhosis. In general, surveillance should only be offered to patients who would consider treatment options for a newly diagnosed HCC and not offered to those with limited life expectancy. The benefits of measuring alpha-fetoprotein concentration in addition to ultrasound remains unclear across a range of liver diseases, including hemochromatosis. Regression of cirrhosis to Scheuer grade F2 or less with phlebotomy therapy is associated with a reduction in the risk of liver cancer, although the majority of cases of cirrhosis persist after treatment [61]. Where such regression is proven, clinicians may consider cessation of surveillance for HCC.

Recommendation

In patients with cirrhosis due to hemochromatosis, six-monthly surveillance with ultrasound, with or without alpha-fetoprotein testing is recommended. Surveillance should only be undertaken if a diagnosis of HCC will alter management (HIGH QUALITY of EVIDENCE; STRONG RECOMMENDATION) (Fig. 2).

Question 15: what is the therapeutic role of hepcidin or hepcidin mimetics?

Venesection therapy for hemochromatosis remains the treatment of choice, with iron depletion preventing organ dysfunction if commenced early. However, compliance and tolerance issues limit its suitability in some patients. Recent evidence on hepcidin modulation in animal models and development of hepcidin mimetics/agonists has demonstrated their potential use in therapy for hemochromatosis and other iron overload disorders.

A recent clinical trial examining the hepcidin mimetic, Rusfertide (PTG-300), for use in hereditary hemochromatosis demonstrated reduction in both transferrin saturation and serum iron during treatment, reduced requirement for phlebotomy during the study period, and resulted in control of hepatic iron concentration [163]. Additional phase 2 trials have been undertaken in both transfusion-dependent and non-transfusion-dependent thalassemia patients [164]. PTG-300 was efficacious in reducing serum iron and transferrin saturation with mild to moderate adverse events. A synthetic endogenous human hepcidin (LJPC-401) also significantly reduced serum iron but the study did not examine tissue iron concentrations. The use of mini-hepcidins in preclinical animal models of non-transfusion-dependent thalassemia are more promising, but there are currently no active clinical trials for mini-hepcidins [165].

Of interest, Chen et al. [166] demonstrated that hepcidin overexpression in animal models of hepatic steatosis results in attenuation of steatosis, indicating that patients with both iron overload and hepatic steatosis may benefit from hepcidin-based therapy.

Recommendation

There are insufficient data to recommend the use of hepcidin-based therapy until further clinical trial data are available (HIGH QUALITY of EVIDENCE; STRONG RECOMMENDATION).

Question 16: are patients with hemochromatosis suitable for liver transplantation and what is the risk of hemochromatosis recurrence after liver transplantation?

Hemochromatosis is a very uncommon indication for liver transplantation comprising only about 1% of all transplants, despite a prevalence of 1:200–400 in Caucasian populations. The discordance between the low number of patients undergoing liver transplantation and the high population prevalence

is due to the variable phenotype of the condition with only a small percentage of patients developing sufficiently high liver iron stores to cause cirrhosis, as well as better clinician and community awareness resulting in early diagnosis and therapy. Many patients who proceed to liver transplantation have associated hepatic co-toxicities, such as alcoholic liver disease, MAFLD, or chronic viral hepatitis [167]. Initially, it was thought that patients with hemochromatosis had more adverse outcomes than other groups. This was largely due to the high number of patients who underwent liver transplantation for complicating hepatocellular carcinoma prior to the development of the Milan criteria developed by Mazzaferro et al., [168], as well as higher rates of infectious and cardiovascular complications [168]. Recent studies have shown similar survival and outcomes compared to other causes of liver disease [167, 169]. There is clear evidence that liver transplantation alleviates the underlying pathophysiological defect in hemochromatosis by restoring hepcidin levels to normal and re-establishing normal iron metabolism [169]. Thus, re-accumulation of iron post-liver transplant is most unusual and alternative causes should be considered if this occurs.

Recommendation

Liver transplantation is an appropriate therapy for patients with decompensated liver disease and /or HCC. The risk of recurrence of hepatic iron loading is very low after liver transplantation and alternative causes should be considered should it occur (HIGH QUALITY of EVIDENCE; STRONG RECOMMENDATION).

Question 17: how should pregnant hemochromatosis patients be managed?

It is important to avoid iron deficiency in subjects with known hemochromatosis undergoing venesection who wish to fall pregnant given its adverse effects on pregnancy outcomes [106]. Thus, iron studies should be closely monitored in this situation and venesection adjusted accordingly. Iron studies should be monitored during pregnancy and clinicians should be aware that pregnancy removes about 1 g of iron from the mother [114]. Thus, phlebotomy is usually ceased during pregnancy without adverse outcomes as the likelihood of rapid iron re-accumulation and associated iron toxicity is very low.

Recommendation

Iron studies should be monitored in hemochromatosis patients wishing to fall pregnant and iron deficiency

avoided. In general, phlebotomy can be ceased during pregnancy with iron studies continuing to be monitored (LOW QUALITY of EVIDENCE; STRONG RECOMMENDATION).

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Declarations

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Ethical approval No ethical approval is required, and these clinical guidelines are based on published data. No animal or human studies were performed for this manuscript.

Informed consent No informed consent was required as no studies involving humans were undertaken. These clinical guidelines are based on published data.

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References

- Anderson GJ, Frazer DM. Current understanding of iron homeostasis. *Am J Clin Nutr*. 2017;106:1559S-1566S
- Bacon BR, Tavill AS. Role of the liver in normal iron metabolism. *Semin Liver Dis*. 1984;4:181-192
- Bridle KR, Frazer DM, Wilkins SJ, Dixon JL, Purdie DM, Crawford DH, et al. Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homeostasis. *Lancet*. 2003;361:669-673
- Rishi G, Wallace DF, Subramaniam VN. Hepcidin: regulation of the master iron regulator. *Biosci Rep*. 2015;35:e00192
- Girelli D, Busti F, Brissot P, Cabantchik I, Muckenthaler MU, Porto G. Hemochromatosis classification: update and recommendations by the BIOIRON Society. *Blood*. 2022;139:3018-3029
- Jazwinska EC, Cullen LM, Busfield F, Pyper WR, Webb SI, Powell LW, et al. Haemochromatosis and HLA-H. *Nat Genet*. 1996;14:249-251
- Brissot P, Moirand R, Jouanolle AM, Guyader D, Le Gall JY, Deugnier Y, et al. A genotypic study of 217 unrelated probands diagnosed as "genetic hemochromatosis" on "classical" phenotypic criteria. *J Hepatol*. 1999;30:588-593
- Carella M, D'Ambrosio L, Totaro A, Grifa A, Valentino MA, Piperno A, et al. Mutation analysis of the HLA-H gene in Italian hemochromatosis patients. *Am J Hum Genet*. 1997;60:828-832
- Papanikolaou G, Samuels ME, Ludwig EH, MacDonald ML, Franchini PL, Dube MP, et al. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. *Nat Genet*. 2004;36:77-82
- Merryweather-Clarke AT, Pointon JJ, Jouanolle AM, Rochette J, Robson KJ. Geography of HFE C282Y and H63D mutations. *Genet Test*. 2000;4:183-198
- Adams PC, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, et al. Hemochromatosis and iron-overload screening in a racially diverse population. *N Engl J Med*. 2005;352:1769-1778
- Barton JC, Edwards CQ, et al. HFE Hemochromatosis. In: Adam MP, Everman DB, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, et al., editors. *GeneReviews*(R). Seattle (WA): University of Washington; 1993
- McDonald CJ, Wallace DF, Crawford DH, Subramaniam VN. Iron storage disease in Asia-Pacific populations: the importance of non-HFE mutations. *J Gastroenterol Hepatol*. 2013;28:1087-1094
- Karimi M, Yavarian M, Delbini P, Hartevelde CL, Farjadian S, Fiorelli G, et al. Spectrum and haplotypes of the HFE hemochromatosis gene in Iran: H63D in beta-thalassemia major and the first E277K homozygous. *Hematol J*. 2004;5:524-527
- Steiner M, Ocran K, Genschel J, Meier P, Gerl H, Ventz M, et al. A homozygous HFE gene splice site mutation (IVS5+1 G/A) in a hereditary hemochromatosis patient of Vietnamese origin. *Gastroenterology*. 2002;122:789-795
- Takano A, Niimi H, Atarashi Y, Sawasaki T, Terasaki T, Nakabayashi T, et al. A novel Y231del mutation of HFE in hereditary haemochromatosis provides in vivo evidence that the Huh-7 is a human haemochromatotic cell line. *Liver Int*. 2011;31:1593-1597
- Lv T, Zhang W, Xu A, Li Y, Zhou D, Zhang B, et al. Non-HFE mutations in haemochromatosis in China: combination of heterozygous mutations involving HJV signal peptide variants. *J Med Genet*. 2018;55:650-660
- Hayashi H, Wakusawa S, Motonishi S, Miyamoto K, Okada H, Inagaki Y, et al. Genetic background of primary iron overload syndromes in Japan. *Intern Med*. 2006;45:1107-1111
- Koyama C, Wakusawa S, Hayashi H, Suzuki R, Yano M, Yoshioka K, et al. Two novel mutations, L490R and V561X, of the transferrin receptor 2 gene in Japanese patients with hemochromatosis. *Haematologica*. 2005;90:302-307
- Lee PL, Halloran C, West C, Beutler E. Mutation analysis of the transferrin receptor-2 gene in patients with iron overload. *Blood Cells Mol Dis*. 2001;27:285-289
- Zhang W, Xu A, Li Y, Zhao S, Zhou D, Wu L, et al. A novel SLC40A1 p.Y333H mutation with gain of function of ferroportin: A recurrent cause of haemochromatosis in China. *Liver Int*. 2019;39:1120-1127
- Lok CY, Merryweather-Clarke AT, Viprakasit V, Chinthammitr Y, Srichairatanakool S, Limwongse C, et al. Iron overload in the Asian community. *Blood*. 2009;114:20-25
- Delatycki MB, Allen KJ, Nisselle AE, Collins V, Metcalfe S, du Sart D, et al. Use of community genetic screening to prevent HFE-associated hereditary haemochromatosis. *Lancet*. 2005;366:314-316
- Allen KJ, Gurrin LC, Constantine CC, Osborne NJ, Delatycki MB, Nicoll AJ, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. *N Engl J Med*. 2008;358:221-230

25. Pilling LC, Tamosauskaite J, Jones G, Wood AR, Jones L, Kuo CL, et al. Common conditions associated with hereditary haemochromatosis genetic variants: cohort study in UK Biobank. *BMJ*. 2019;364: k5222
26. Distant S, Berg JP, Lande K, Haug E, Bell H. HFE gene mutation (C282Y) and phenotypic expression among a hospitalised population in a high prevalence area of haemochromatosis. *Gut*. 2000;47:575–579
27. Sanchez M, Villa M, Ingelmo M, Sanz C, Bruguera M, Ascaso C, et al. Population screening for hemochromatosis: a study in 5370 Spanish blood donors. *J Hepatol*. 2003;38:745–750
28. Deugnier Y, Jouanolle AM, Chaperon J, Moirand R, Pithois C, Meyer JF, et al. Gender-specific phenotypic expression and screening strategies in C282Y-linked haemochromatosis: a study of 9396 French people. *Br J Haematol*. 2002;118:1170–1178
29. Trousseau A. Glycosurie; diabete sucre. *Clinique Med de l'Hotel-dieu de Paris (2nd ed)* 1865;2:663–698
30. von Recklinghausen FD. *Über haemochromatose*. *Tageblatt der (62) Versammlung Deutscher Naturforscher und Ärzte Heidelberg 1889*;62:324–325
31. Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, Strohmeyer G. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. *N Engl J Med*. 1985;313:1256–1262
32. Sheldon JH. *Haemochromatosis*. London: Oxford University Press; 1935
33. Simon M, Alexandre JL, Bourel M, Le Marec B, Scordia C. Heredity of idiopathic haemochromatosis: a study of 106 families. *Clin Genet*. 1977;11:327–341
34. Niederau C, Fischer R, Purschel A, Stremmel W, Haussinger D, Strohmeyer G. Long-term survival in patients with hereditary hemochromatosis. *Gastroenterology*. 1996;110:1107–1119
35. Simon M, Bourel M, Genetet B, Fauchet R. Idiopathic hemochromatosis. Demonstration of recessive transmission and early detection by family HLA typing. *N Engl J Med*. 1977;297:1017–1021
36. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet*. 1996;13:399–408
37. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of 845G→A (C282Y) HFE hereditary haemochromatosis mutation in the USA. *Lancet*. 2002;359:211–218
38. Olynyk JK, Cullen DJ, Aquilia S, Rossi E, Summerville L, Powell LW. A population-based study of the clinical expression of the hemochromatosis gene. *N Engl J Med*. 1999;341:718–724
39. Coppin H, Bensaid M, Fruchon S, Borot N, Blanche H, Roth MP. Longevity and carrying the C282Y mutation for haemochromatosis on the HFE gene: case control study of 492 French centenarians. *BMJ*. 2003;327:132–133
40. Piippo K, Louhija J, Tilvis R, Kontula K. You may live to the age of more than 100 years even if you are homozygous for a haemochromatosis gene mutation. *Eur J Clin Invest*. 2003;33:830–831
41. Van Aken MO, De Craen AJ, Gussekloo J, Moghaddam PH, Vandenbroucke JP, Heijmans BT, et al. No increase in mortality and morbidity among carriers of the C282Y mutation of the hereditary haemochromatosis gene in the oldest old: the Leiden 85-plus study. *Eur J Clin Invest*. 2002;32:750–754
42. Willis G, Wimperis JZ, Smith K, Fellows IW, Jennings BA. HFE mutations in the elderly. *Blood Cells Mol Dis*. 2003;31:240–246
43. Atkins JL, Pilling LC, Masoli JAH, Kuo CL, Shearman JD, Adams PC, et al. Association of hemochromatosis HFE p.C282Y homozygosity with hepatic malignancy. *JAMA*. 2020;324:2048–2057
44. Waalen J, Felitti V, Gelbart T, Ho NJ, Beutler E. Penetrance of hemochromatosis. *Blood Cells Mol Dis*. 2002;29:418–432
45. Atkins JL, Pilling LC, Heales CJ, Savage S, Kuo CL, Kuchel GA, et al. Hemochromatosis mutations, brain iron imaging, and dementia in the UK Biobank Cohort. *J Alzheimers Dis*. 2021;79:1203–1211
46. Tamosauskaite J, Atkins JL, Pilling LC, Kuo CL, Kuchel GA, Ferrucci L, et al. Hereditary hemochromatosis associations with frailty, sarcopenia and chronic pain: evidence from 200,975 older UK Biobank participants. *J Gerontol A Biol Sci Med Sci*. 2019;74:337–342
47. Powell LW, Dixon JL, Ramm GA, Purdie DM, Lincoln DJ, Anderson GJ, et al. Screening for hemochromatosis in asymptomatic subjects with or without a family history. *Arch Intern Med*. 2006;166:294–301
48. Wojcik JP, Speechley MR, Kertesz AE, Chakrabarti S, Adams PC. Natural history of C282Y homozygotes for hemochromatosis. *Can J Gastroenterol*. 2002;16:297–302
49. Anderson GJ, Bardou-Jacquet E. Revisiting hemochromatosis: genetic vs. phenotypic manifestations. *Ann Transl Med* 2021;9:731
50. Warne CD, Zaloumis SG, Bertalli NA, Delatycki MB, Nicoll AJ, McLaren CE, et al. HFE p.C282Y homozygosity predisposes to rapid serum ferritin rise after menopause: a genotype-stratified cohort study of hemochromatosis in Australian women. *J Gastroenterol Hepatol*. 2017;32:797–802
51. Bacon BR, Adams PC, Kowdley KV, Powell LW, Tavill AS, American Association for the Study of Liver D. Diagnosis and management of hemochromatosis: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology*. 2011;54:328–343
52. Kowdley KV, Brown KE, Ahn J, Sundaram V. ACG clinical guideline: hereditary hemochromatosis. *Am J Gastroenterol*. 2019;114:1202–1218
53. Osborne NJ, Gurrin LC, Allen KJ, Constantine CC, Delatycki MB, McLaren CE, et al. HFE C282Y homozygotes are at increased risk of breast and colorectal cancer. *Hepatology*. 2010;51:1311–1318
54. Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. *J Hepatol*. 1991;13:372–374
55. Bacon BR, Olynyk JK, Brunt EM, Britton RS, Wolff RK. HFE genotype in patients with hemochromatosis and other liver diseases. *Ann Intern Med*. 1999;130:953–962
56. Chin J, Powell LW, Ramm LE, Ayonrinde OT, Ramm GA, Olynyk JK. Utility of hepatic or total body iron burden in the assessment of advanced hepatic fibrosis in HFE hemochromatosis. *Sci Rep*. 2019;9:20234
57. Andersson L, Powell LW, Ramm LE, Ramm GA, Olynyk JK. Arthritis prediction of advanced hepatic fibrosis in HFE hemochromatosis. *Mayo Clin Proc*. 2022;97:1649–1655
58. Beaton M, Guyader D, Deugnier Y, Moirand R, Chakrabarti S, Adams P. Noninvasive prediction of cirrhosis in C282Y-linked hemochromatosis. *Hepatology*. 2002;36:673–678
59. Wood MJ, Crawford DHG, Wockner LF, Powell LW, Ramm GA. Serum ferritin concentration predicts hepatic fibrosis better than hepatic iron concentration in human HFE-Haemochromatosis. *Liver Int*. 2017;37:1382–1388
60. Wood MJ, Powell LW, Dixon JL, Ramm GA. Clinical cofactors and hepatic fibrosis in hereditary hemochromatosis: the role of diabetes mellitus. *Hepatology*. 2012;56:904–911
61. Bardou-Jacquet E, Morandau E, Anderson GJ, Ramm GA, Ramm LE, Morcet J, et al. Regression of fibrosis stage with treatment reduces long-term risk of liver cancer in patients with hemochromatosis caused by mutation in HFE. *Clin Gastroenterol Hepatol*. 2020;18:1851–1857
62. Brissot P, Pietrangelo A, Adams PC, de Graaff B, McLaren CE, Loreal O. Haemochromatosis. *Nat Rev Dis Primers*. 2018;4:18016

63. Adams PC, Speechley M. The effect of arthritis on the quality of life in hereditary hemochromatosis. *J Rheumatol.* 1996;23:707–710
64. Carroll GJ, Bredahl WH, Bulsara MK, Olynyk JK. Hereditary hemochromatosis is characterized by a clinically definable arthropathy that correlates with iron load. *Arthritis Rheum.* 2011;63:286–294
65. Bardou-Jacquet E, Laine F, Guggenbuhl P, Morcet J, Jezequel C, Guyader D, et al. Worse outcomes of patients with HFE hemochromatosis with persistent increases in transferrin saturation during maintenance therapy. *Clin Gastroenterol Hepatol.* 2017;15:1620–1627
66. Carroll GJ, Bredahl WH, Olynyk JK. Characteristics of the arthropathy described in hereditary hemochromatosis. *Arthritis Care Res (Hoboken).* 2012;64:9–14
67. Altes A, Ruiz A, Martinez C, Esteve A, Vela MD, Remacha AF, et al. The relationship between iron overload and clinical characteristics in a Spanish cohort of 100 C282Y homozygous hemochromatosis patients. *Ann Hematol.* 2007;86:831–835
68. Valenti L, Fracanzani AL, Rossi V, Rampini C, Pulixi E, Varenna M, et al. The hand arthropathy of hereditary hemochromatosis is strongly associated with iron overload. *J Rheumatol.* 2008;35:153–158
69. Pietrangelo A. Hereditary hemochromatosis: pathogenesis, diagnosis, and treatment. *Gastroenterology.* 2010;139:393–408
70. Eslam M, Sanyal AJ, George J, International Consensus P. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology.* 2020;158:1999–2014e1991
71. Kowdley KV. Iron overload in patients with chronic liver disease. *Gastroenterol Hepatol (N Y).* 2016;12:695–698
72. Harrison-Findik DD, Schafer D, Klein E, Timchenko NA, Kulaksiz H, Clemens D, et al. Alcohol metabolism-mediated oxidative stress down-regulates hepcidin transcription and leads to increased duodenal iron transporter expression. *J Biol Chem.* 2006;281:22974–22982
73. Fletcher LM, Dixon JL, Purdie DM, Powell LW, Crawford DH. Excess alcohol greatly increases the prevalence of cirrhosis in hereditary hemochromatosis. *Gastroenterology.* 2002;122:281–289
74. Deugnier Y, Bardou-Jacquet E, Laine F. Dysmetabolic iron overload syndrome. *Bull Acad Natl Med.* 2016;200:327–333
75. Ramm GA, Crawford DH, Powell LW, Walker NI, Fletcher LM, Halliday JW. Hepatic stellate cell activation in genetic haemochromatosis. Lobular distribution, effect of increasing hepatic iron and response to phlebotomy. *J Hepatol.* 1997;26:584–592
76. Ramm GA, Ruddell RG. Hepatotoxicity of iron overload: mechanisms of iron-induced hepatic fibrogenesis. *Semin Liver Dis.* 2005;25:433–449
77. Ramm GA, Ruddell RG. Iron homeostasis, hepatocellular injury, and fibrogenesis in hemochromatosis: the role of inflammation in a noninflammatory liver disease. *Semin Liver Dis.* 2010;30:271–287
78. Rishi G, Huang G, Subramaniam VN. Cancer: the role of iron and ferroptosis. *Int J Biochem Cell Biol.* 2021;141: 106094
79. Wu J, Wang Y, Jiang R, Xue R, Yin X, Wu M, et al. Ferroptosis in liver disease: new insights into disease mechanisms. *Cell Death Discov.* 2021;7:276
80. Bacon BR, Britton RS. The pathology of hepatic iron overload: a free radical-mediated process? *Hepatology.* 1990;11:127–137
81. Bacon BR, Park CH, Brittenham GM, O'Neill R, Tavill AS. Hepatic mitochondrial oxidative metabolism in rats with chronic dietary iron overload. *Hepatology.* 1985;5:789–797
82. Bacon BR, Tavill AS, Brittenham GM, Park CH, Recknagel RO. Hepatic lipid peroxidation in vivo in rats with chronic iron overload. *J Clin Invest.* 1983;71:429–439
83. Pietrangelo A. Iron-induced oxidant stress in alcoholic liver fibrogenesis. *Alcohol.* 2003;30:121–129
84. Ruddell RG, Hoang-Le D, Barwood JM, Rutherford PS, Piva TJ, Watters DJ, et al. Ferritin functions as a proinflammatory cytokine via iron-independent protein kinase C zeta/nuclear factor kappaB-regulated signaling in rat hepatic stellate cells. *Hepatology.* 2009;49:887–900
85. Bloomer SA, Brown KE. Iron-induced liver injury: a critical reappraisal. *Int J Mol Sci.* 2019;20:2132
86. Bridle KR, Crawford DH, Ramm GA. Identification and characterization of the hepatic stellate cell transferrin receptor. *Am J Pathol.* 2003;162:1661–1667
87. Gardi C, Arezzini B, Fortino V, Comporti M. Effect of free iron on collagen synthesis, cell proliferation and MMP-2 expression in rat hepatic stellate cells. *Biochem Pharmacol.* 2002;64:1139–1145
88. Mehta KJ, Coombes JD, Briones-Orta M, Manka PP, Williams R, Patel VB, et al. Iron enhances hepatic fibrogenesis and activates transforming growth factor-beta signaling in murine hepatic stellate cells. *Am J Med Sci.* 2018;355:183–190
89. Brown KE, Poulos JE, Li L, Soweid AM, Ramm GA, O'Neill R, et al. Effect of vitamin E supplementation on hepatic fibrogenesis in chronic dietary iron overload. *Am J Physiol.* 1997;272:G116–123
90. Ramm GA, Li SC, Li L, Britton RS, O'Neill R, Kobayashi Y, et al. Chronic iron overload causes activation of rat lipocytes in vivo. *Am J Physiol.* 1995;268:G451–458
91. Wood MJ, Gadd VL, Powell LW, Ramm GA, Clouston AD. Ductular reaction in hereditary hemochromatosis: the link between hepatocyte senescence and fibrosis progression. *Hepatology.* 2014;59:848–857
92. Lowes KN, Brennan BA, Yeoh GC, Olynyk JK. Oval cell numbers in human chronic liver diseases are directly related to disease severity. *Am J Pathol.* 1999;154:537–541
93. Ramm GA, Britton RS, O'Neill R, Bacon BR. Identification and characterization of a receptor for tissue ferritin on activated rat lipocytes. *J Clin Invest.* 1994;94:9–15
94. Powell LW, Jazwinska E, Halliday JW. Primary iron overload. In Brock J, Halliday J, Pippard M, Powell L, editors., *Iron metabolism in health and disease*, vol. 227270. Philadelphia: WB Saunders and Co., Ltd.; 1994
95. Olynyk JK, Ramm GA. Hemochromatosis. *N Engl J Med.* 2022;387:2159–2170
96. Wood MJ, Powell LW, Ramm GA. Environmental and genetic modifiers of the progression to fibrosis and cirrhosis in hemochromatosis. *Blood.* 2008;111:4456–4462
97. Pelucchi S, Galimberti S, Greni F, Rametta R, Mariani R, Pelloni I, et al. Proprotein convertase 7 rs236918 associated with liver fibrosis in Italian patients with HFE-related hemochromatosis. *J Gastroenterol Hepatol.* 2016;31:1342–1348
98. Stickel F, Buch S, Zoller H, Hultcrantz R, Gallati S, Osterreicher C, et al. Evaluation of genome-wide loci of iron metabolism in hereditary hemochromatosis identifies PCSK7 as a host risk factor of liver cirrhosis. *Hum Mol Genet.* 2014;23:3883–3890
99. Valenti L, Maggioni P, Piperno A, Rametta R, Pelucchi S, Mariani R, et al. Patatin-like phospholipase domain containing-3 gene I148M polymorphism, steatosis, and liver damage in hereditary hemochromatosis. *World J Gastroenterol.* 2012;18:2813–2820
100. Jacobs EM, Hendriks JC, Marx JJ, van Deursen CT, Kreeftenberg HG, de Vries RA, et al. Morbidity and mortality in first-degree relatives of C282Y homozygous probands with clinically detected haemochromatosis compared with the general

- population: the HEMochromatosis FAmily Study (HEFAS). *Neth J Med*. 2007;65:425–433
101. Whitlock EP, Garlitz BA, Harris EL, Beil TL, Smith PR. Screening for hereditary hemochromatosis: a systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med*. 2006;145:209–223
 102. Adams PC. Implications of genotyping of spouses to limit investigation of children in genetic hemochromatosis. *Clin Genet*. 1998;53:176–178
 103. Delatycki MB, Wolthuizen M, Collins V, Varley E, Craven J, Allen KJ, et al. ironXS: high-school screening for hereditary haemochromatosis is acceptable and feasible. *Eur J Hum Genet*. 2012;20:505–509
 104. Adams P, Barton JC, McLaren GD, Acton RT, Speechley M, McLaren CE, et al. Screening for iron overload: lessons from the hemochromatosis and iron overload screening (HEIRS) study. *Can J Gastroenterol*. 2009;23:769–772
 105. Nadakkavukaran IM, Gan EK, Olynyk JK. Screening for hereditary haemochromatosis. *Pathology*. 2012;44:148–152
 106. European Association for the Study of the Liver. Electronic address eee, European Association for the Study of the L. EASL Clinical Practice Guidelines on haemochromatosis. *J Hepatol*. 2022;77:479–502
 107. Galhenage SP, Viiala CH, Olynyk JK. Screening for hemochromatosis: patients with liver disease, families, and populations. *Curr Gastroenterol Rep*. 2004;6:44–51
 108. Adams PC, Reboussin DM, Press RD, Barton JC, Acton RT, Moses GC, et al. Biological variability of transferrin saturation and unsaturated iron-binding capacity. *Am J Med*. 2007;120(999):e991–997
 109. Wong K, Adams PC. The diversity of liver diseases among outpatient referrals for an elevated serum ferritin. *Can J Gastroenterol*. 2006;20:467–470
 110. Leggett BA, Brown NN, Bryant SJ, Duplock L, Powell LW, Halliday JW. Factors affecting the concentrations of ferritin in serum in a healthy Australian population. *Clin Chem*. 1990;36:1350–1355
 111. Ong SY, Nicoll AJ, Delatycki MB. How should hyperferritinaemia be investigated and managed? *Eur J Intern Med*. 2016;33:21–27
 112. Australia TRCoPo. Ferritin. <https://www.rcpa.edu.au/Manuals/RCPA-Manual/Pathology-Tests/F/Ferritin>; 2022. Accessed 22 Feb 2023
 113. McKinnon EJ, Rossi E, Beilby JP, Trinder D, Olynyk JK. Factors that affect serum levels of ferritin in Australian adults and implications for follow-up. *Clin Gastroenterol Hepatol*. 2014;12(101–108): e104
 114. Powell LW, Seckington RC, Deugnier Y. Haemochromatosis. *Lancet*. 2016;388:706–716
 115. Olynyk JK, St Pierre TG, Britton RS, Brunt EM, Bacon BR. Duration of hepatic iron exposure increases the risk of significant fibrosis in hereditary hemochromatosis: a new role for magnetic resonance imaging. *Am J Gastroenterol*. 2005;100:837–841
 116. Gandon Y, Olivie D, Guyader D, Aube C, Oberti F, Sebille V, et al. Non-invasive assessment of hepatic iron stores by MRI. *Lancet*. 2004;363:357–362
 117. Garbowski MW, Carpenter JP, Smith G, Roughton M, Alam MH, He T, et al. Biopsy-based calibration of T2* magnetic resonance for estimation of liver iron concentration and comparison with R2 Ferriscan. *J Cardiovasc Magn Reson*. 2014;16:40
 118. Hernando D, Cook RJ, Qazi N, Longhurst CA, Diamond CA, Reeder SB. Complex confounder-corrected R2* mapping for liver iron quantification with MRI. *Eur Radiol*. 2021;31:264–275
 119. St Pierre TG, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood*. 2005;105:855–861
 120. Wood JC, Enriquez C, Ghugre N, Tyzka JM, Carson S, Nelson MD, et al. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. *Blood*. 2005;106:1460–1465
 121. Emond MJ, Bronner MP, Carlson TH, Lin M, Labbe RF, Kowdley KV. Quantitative study of the variability of hepatic iron concentrations. *Clin Chem*. 1999;45:340–346
 122. Sarigianni M, Liakos A, Vlachaki E, Paschos P, Athanasiadou E, Montori VM, et al. Accuracy of magnetic resonance imaging in diagnosis of liver iron overload: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. 2015;13(55–63): e55
 123. Anderson LJ, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. *Eur Heart J*. 2001;22:2171–2179
 124. Hernando D, Levin YS, Sirlin CB, Reeder SB. Quantification of liver iron with MRI: state of the art and remaining challenges. *J Magn Reson Imaging*. 2014;40:1003–1021
 125. Quinn CT, St Pierre TG. MRI measurements of iron load in transfusion-dependent patients: implementation, challenges, and pitfalls. *Pediatr Blood Cancer*. 2016;63:773–780
 126. St Pierre TG, El-Beshlawy A, Elalfy M, Al Jefri A, Al Zir K, Daar S, et al. Multicenter validation of spin-density projection-assisted R2-MRI for the noninvasive measurement of liver iron concentration. *Magn Reson Med*. 2014;71:2215–2223
 127. St Pierre TG, Jeffrey GP, Olynyk JK, Pootrakul P. The effect of liver steatosis on non-invasive measurement of liver iron concentration by spin-density projection assisted R2-MRI (FerriScan®). *J Hepatol*. 2017;66:S675–S676
 128. Bassett ML, Halliday JW, Powell LW. Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis. *Hepatology*. 1986;6:24–29
 129. Falize L, Guillygomarc'h A, Perrin M, Laine F, Guyader D, Brissot P, et al. Reversibility of hepatic fibrosis in treated genetic hemochromatosis: a study of 36 cases. *Hepatology*. 2006;44:472–477
 130. Adams PC. Is there a threshold of hepatic iron concentration that leads to cirrhosis in C282Y hemochromatosis? *Am J Gastroenterol*. 2001;96:567–569
 131. Guyader D, Jacquelinet C, Moirand R, Turlin B, Mendler MH, Chaperon J, et al. Noninvasive prediction of fibrosis in C282Y homozygous hemochromatosis. *Gastroenterology*. 1998;115:929–936
 132. Morrison ED, Brandhagen DJ, Phatak PD, Barton JC, Krawitt EL, El-Serag HB, et al. Serum ferritin level predicts advanced hepatic fibrosis among U.S. patients with phenotypic hemochromatosis. *Ann Intern Med*. 2003;138:627–633
 133. Crawford DH, Murphy TL, Ramm LE, Fletcher LM, Clouston AD, Anderson GJ, et al. Serum hyaluronic acid with serum ferritin accurately predicts cirrhosis and reduces the need for liver biopsy in C282Y hemochromatosis. *Hepatology*. 2009;49:418–425
 134. Legros L, Bardou-Jacquet E, Latournerie M, Guillygomarc'h A, Turlin B, Le Lan C, et al. Non-invasive assessment of liver fibrosis in C282Y homozygous HFE hemochromatosis. *Liver Int*. 2015;35:1731–1738
 135. d'Assignies G, Paisant A, Bardou-Jacquet E, Boulic A, Bannier E, Laine F, et al. Non-invasive measurement of liver iron concentration using 3-Tesla magnetic resonance imaging: validation against biopsy. *Eur Radiol*. 2018;28:2022–2030
 136. Franca M, Marti-Bonmati L, Silva S, Oliveira C, Alberich Bayarri A, Vilas Boas F, et al. Optimizing the management of hereditary haemochromatosis: the value of MRI R2*

- quantification to predict and monitor body iron stores. *Br J Haematol.* 2018;183:491–493
137. Anstee QM, Castera L, Loomba R. Impact of non-invasive biomarkers on hepatology practice: Past, present and future. *J Hepatol.* 2022;76:1362–1378
 138. Adams LA, Bulsara M, Rossi E, DeBoer B, Speers D, George J, et al. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. *Clin Chem.* 2005;51:1867–1873
 139. Huang Y, Adams LA, Joseph J, Bulsara MK, Jeffrey GP. The ability of Hepascore to predict liver fibrosis in chronic liver disease: a meta-analysis. *Liver Int.* 2017;37:121–131
 140. Ong SY, Khoo T, Nicoll AJ, Gurrin L, Worland T, Pateria P, et al. Utility and limitations of Hepascore and transient elastography to detect advanced hepatic fibrosis in HFE hemochromatosis. *Sci Rep.* 2021;11:14654
 141. Chin J, Powell LW, Ramm LE, Hartel GF, Olynyk JK, Ramm GA. Utility of serum biomarker indices for staging of hepatic fibrosis before and after venesection in patients with hemochromatosis caused by variants in HFE. *Clin Gastroenterol Hepatol.* 2021;19(1459–1468): e1455
 142. Gurrin LC, Osborne NJ, Constantine CC, McLaren CE, English DR, Gertig DM, et al. The natural history of serum iron indices for HFE C282Y homozygosity associated with hereditary hemochromatosis. *Gastroenterology.* 2008;135:1945–1952
 143. Powell EE, Ali A, Clouston AD, Dixon JL, Lincoln DJ, Purdie DM, et al. Steatosis is a cofactor in liver injury in hemochromatosis. *Gastroenterology.* 2005;129:1937–1943
 144. Ong SY, Gurrin LC, Dolling L, Dixon J, Nicoll AJ, Wolthuizen M, et al. Reduction of body iron in HFE-related hemochromatosis and moderate iron overload (Mi-Iron): a multicentre, participant-blinded, randomised controlled trial. *Lancet Haematol.* 2017;4:e607–e614
 145. Hicken BL, Tucker DC, Barton JC. Patient compliance with phlebotomy therapy for iron overload associated with hemochromatosis. *Am J Gastroenterol.* 2003;98:2072–2077
 146. Adams PC. Factors affecting the rate of iron mobilization during venesection therapy for genetic hemochromatosis. *Am J Hematol.* 1998;58:16–19
 147. Rombout-Sestrienkova E, Brandts L, Koek GH, van Deursen C. Patients with hereditary hemochromatosis reach safe range of transferrin saturation sooner with erythrocytaphereses than with phlebotomies. *J Clin Apher.* 2022;37:100–105
 148. Rombout-Sestrienkova E, Koek GH, Neslo R, van Kraaij M, Menheere PP, Masclee A, et al. Course of iron parameters in HFE-hemochromatosis patients during initial treatment with erythrocytapheresis compared to phlebotomy. *J Clin Apher.* 2016;31:564–570
 149. Rombout-Sestrienkova E, Winkens B, Essers BA, Nieman FH, Noord PA, Janssen MC, et al. Erythrocytapheresis versus phlebotomy in the maintenance treatment of HFE hemochromatosis patients: results from a randomized crossover trial. *Transfusion.* 2016;56:261–270
 150. Rombout-Sestrienkova E, van Kraaij MG, Koek GH. How we manage patients with hereditary haemochromatosis. *Br J Haematol.* 2016;175:759–770
 151. Cancado R, Melo MR, de Moraes BR, Santos PC, Guerra-Shinohara EM, Chiattonne C, et al. Deferasirox in patients with iron overload secondary to hereditary hemochromatosis: results of a 1-yr Phase 2 study. *Eur J Haematol.* 2015;95:545–550
 152. Lescano MA, Tavares LC, Santos P. Juvenile hemochromatosis: HAMP mutation and severe iron overload treated with phlebotomies and deferasirox. *World J Clin Cases.* 2017;5:381–383
 153. Sandhu K, Flintoff K, Chatfield MD, Dixon JL, Ramm LE, Ramm GA, et al. Phenotypic analysis of hemochromatosis subtypes reveals variations in severity of iron overload and clinical disease. *Blood.* 2018;132:101–110
 154. Kaltwasser JP, Werner E, Schalk K, Hansen C, Gottschalk R, Seidl C. Clinical trial on the effect of regular tea drinking on iron accumulation in genetic haemochromatosis. *Gut.* 1998;43:699–704
 155. Milward EA, Baines SK, Knuiman MW, Bartholomew HC, Divitini ML, Ravine DG, et al. Noncitrus fruits as novel dietary environmental modifiers of iron stores in people with or without HFE gene mutations. *Mayo Clin Proc.* 2008;83:543–549
 156. Nienhuis AW. Vitamin C and iron. *N Engl J Med.* 1981;304:170–171
 157. Barton JC, Acton RT. Hemochromatosis and *Vibrio vulnificus* wound infections. *J Clin Gastroenterol.* 2009;43:890–893
 158. Horseman MA, Surani S. A comprehensive review of *Vibrio vulnificus*: an important cause of severe sepsis and skin and soft-tissue infection. *Int J Infect Dis.* 2011;15:e157-166
 159. Kanwal F, Singal AG. Surveillance for hepatocellular carcinoma: current best practice and future direction. *Gastroenterology.* 2019;157:54–64
 160. Singal AG, Pillai A, Tiro J. Early detection, curative treatment, and survival rates for hepatocellular carcinoma surveillance in patients with cirrhosis: a meta-analysis. *PLoS Med.* 2014;11: e1001624
 161. Chinnaratha MA, Campbell K, Mathias R, McCormick RJ, Woodman RJ, Wigg AJ. Improved survival of hepatocellular carcinoma patients diagnosed with a dedicated screening programme—a propensity score adjusted analysis. *J Gastrointest Cancer.* 2019;50:888–893
 162. Bertot LC, Jeffrey GP, Wallace M, MacQuillan G, Garas G, Ching HL, et al. Nonalcoholic fatty liver disease-related cirrhosis is commonly unrecognized and associated with hepatocellular carcinoma. *Hepatol Commun.* 2017;1:53–60
 163. Kowdley KV, Modi N, Valone F, Priego V, Cole F, Pouzar J, et al. An open-label phase 2, dose-finding study of the safety and efficacy of Rusfertide (PTG-300), a hepcidin mimetic, in patients with hereditary hemochromatosis. *Hepatology.* 2021;74:24A
 164. Lal A, Voskaridou E, Flevari P, Taher A, Chew L-P, Valone F, Gupta S, et al. A hepcidin mimetic, PTG-300, demonstrates pharmacodynamic effects indicating reduced iron availability in transfusion-dependent beta-thalassemia subjects. In: 25th European Hematology Association Annual Congress; 2020; Tokyo, Japan; 2020. 4(S1):S298
 165. Longo F, Piga A. Does hepcidin tuning have a role among emerging treatments for thalassemia? *J Clin Med.* 2022;11(17):5119
 166. Chen H, Zhao W, Yan X, Huang T, Yang A. Overexpression of hepcidin alleviates steatohepatitis and fibrosis in a diet-induced nonalcoholic steatohepatitis. *J Clin Transl Hepatol.* 2022;10:577–588
 167. Crawford DH, Fletcher LM, Hubscher SG, Stuart KA, Gane E, Angus PW, et al. Patient and graft survival after liver transplantation for hereditary hemochromatosis: Implications for pathogenesis. *Hepatology.* 2004;39:1655–1662
 168. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med.* 1996;334:693–699
 169. Bardou-Jacquet E, Philip J, Lorho R, Ropert M, Latournerie M, Housset-Debry P, et al. Liver transplantation normalizes serum hepcidin level and cures iron metabolism alterations in HFE hemochromatosis. *Hepatology.* 2014;59:839–847

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