Clinical Practice Guidelines

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EASL Clinical Practice Guidelines on haemochromatosis☆

European Association for the Study of the Liver*

Summary

Haemochromatosis is characterised by elevated transferrin saturation (TSAT) and progressive iron loading that mainly affects the liver. Early diagnosis and treatment by phlebotomy can prevent cirrhosis, hepatocellular carcinoma, diabetes, arthropathy and other complications. In patients homozygous for p.Cys282Tyr in *HFE*, provisional iron overload based on serum iron parameters (TSAT >45% and ferritin >200 μg/L in females and TSAT >50% and ferritin >300 μg/L in males and postmenopausal women) is sufficient to diagnose haemochromatosis. In patients with high TSAT and elevated ferritin but other *HFE* genotypes, diagnosis requires the presence of hepatic iron overload on MRI or liver biopsy. The stage of liver fibrosis and other end-organ damage should be carefully assessed at diagnosis because they determine disease management. Patients with advanced fibrosis should be included in a screening programme for hepatocellular carcinoma. Treatment targets for phlebotomy are ferritin <50 μg/L during the induction phase and <100 μg/L during the maintenance phase.

Introduction

Haemochromatosis is a disorder of iron homeostasis that is characterised by increased intestinal iron absorption and iron release from macrophages, leading to an expanded circulating iron pool, reflected by increased transferrin saturation (Box 1). This can lead to progressive body iron accumulation, that mainly manifests in the liver. If untreated, haemochromatosis can result in liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC).[1,2] Common manifestations also include diabetes, osteoporosis and arthropathy.[3] In patients with severe or early onset haemochromatosis, the disease can be associated with hypogonadotrophic hypogonadism, hypothyroidism and heart failure.[4] Typical symptoms and signs of haemochromatosis include weakness, fatigue and a greyish-brown discoloration of the skin. Males are affected significantly more frequently than females and disease prevalence increases with age.[5]

Haemochromatosis is an autosomal recessive condition and homozygosity for the p.Cys282Tyr (p.C282Y) variant in *HFE* is present in about 80% of individuals of European origin with the disease.[6] This genotype has a variable prevalence in Europe ranging from 1:83 in Ireland to less than 1:2,500 in Southern Europe.[7] Rarely, haemochromatosis is caused by recessive pathogenic variants in genes encoding the iron hormone hepcidin (*HAMP*), transferrin receptor 2 (*TFR2*) or hemojuvelin (*HJV*), or gain of function dominant variants in the gene encoding ferroportin (*SLC40A1*), but the identification of the specific disease-causing genetic variant is neither required in patients with adult-onset haemochromatosis nor sufficient for the diagnosis of haemochromatosis, which is based on phenotypic criteria. Individuals who are homozygous for the p.C282Y variant in *HFE* are at risk of developing haemochromatosis, but the exact disease penetrance is dependent on age and sex. A large follow-up study over a mean period of 12 years in 203 p.C282Y homozygotes between 40 and 69 years of age showed that the penetrance of iron overload-related disease was 28% (95% CI 18-40%) in men and 1.2% (95% CI 0.03-6.5%) in women; in the same study 81.8% of men and 55.4% of women had increased serum ferritin concentrations at baseline, suggesting higher rates of biochemical penetrance.[8]

Elevated transferrin saturation and high serum ferritin should prompt *HFE* genotyping as homozygosity for p.C282Y can confirm the diagnosis of haemochromatosis.[9] In patients with elevated transferrin saturation who are not homozygous for p.C282Y, non-invasive quantification of hepatic iron with MRI is recommended, because patients with haemochromatosis typically have high concentrations of iron in the liver.[10] If available, spleen iron quantification by MRI can provide useful additional diagnostic information, because spleen iron is low or normal in haemochromatosis.[10] Important differential diagnoses for haemochromatosis include alcohol- or metabolic-dysfunction associated fatty liver disease, and iron-loading anaemias, such as beta-thalassemias, dyserythropoietic anaemias, and ferroportin disease (caused by heterozygous pathogenic loss of function variants in the *SLC40A1* gene). Other differential diagnoses include rare iron-storage disorders such as aceruloplasminemia or atransferrinemia, which are not covered in this guideline.

Disease management is determined by organ involvement and liver disease stage. At early stages, therapeutic phlebotomy will improve fatigue in most patients, prevent or halt progression of liver fibrosis and normalise life expectancy.[11,12] At advanced disease stages, phlebotomy can induce fibrosis regression and can even cause regression of early cirrhosis.[13] In contrast, phlebotomy has not been shown to be of benefit and is not recommended in patients with high ferritin or iron

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Box 1. Key background information.

Definition: Haemochromatosis is a disease of genetic origin characterised by increased transferrin saturation and liver iron overload, in the absence of anaemia and/or reticulocytosis. At early disease stages, hepatic iron deposition primarily affects peri-portal hepatocytes, but not Kupffer cells. Spleen iron overload is typically absent.

Pathogenesis: Haemochromatosis is caused by a deficiency or impairment in hepcidin synthesis or function* due to pathogenic variants in genes regulating hepcidin production or function, which leads to increased intestinal iron absorption and iron release from macrophages resulting in an expanded circulating iron pool, reflected by increased transferrin saturation, which can lead to progressive body iron accumulation, mainly in the liver.

Genetics: Homozygosity for the p.Cys282Tyr variant in HFE is by far the commonest genetic variant predisposing to haemochromatosis in individuals of European origin. In individuals of non-European origin, or in individuals of European origin who are not homozygous for the p.Cys282Tyr variant in HFE, haemochromatosis is caused by rarer variants in the HFE or non-HFE-haemochromatosis genes.

Specification: Transferrin saturation can also be increased in patients with advanced cirrhosis, low transferrin, acute liver failure, acute liver injury, and may be confused with a predisposition to haemochromatosis.

*Despite the relevance of hepcidin in the pathogenesis of haemochromatosis, its measurement is not necessary for diagnosis, as persistently increased transferrin saturation is a sufficiently specific marker of hepcidin deficiency.

overload linked to metabolic-dysfunction associated liver disease and not haemochromatosis.[14,15] Correct diagnosis, adequate treatment and appropriate follow-up are therefore essential in the care and prevention of complications in patients with haemochromatosis.

The present clinical practice guidelines (CPGs) were developed to guide healthcare professionals, patients, their relatives, researchers, policy makers and other stakeholders on the diagnosis and management of haemochromatosis.

Methods

These CPGs were developed by a panel of experts chosen and approved by the EASL Governing Board. The development process followed a standardised method that complies with the international standards for CPGs according to the guidelines international network.[16]

The guidelines were developed during weekly videoconferences among the experts in 2021 and the evidence and recommendations in these guidelines have been graded according to the OCEBM Levels of Evidence Working Group system (Table 1).[17] The strength of recommendations (strong: 1, weak: 2) thus reflects the quality (grade) of underlying evidence (1,2,3,4,5). Grades are not provided for statements and definitions.

The panellists were responsible for making an unbiased selection of literature to determine the level of evidence for their recommendations. Besides the evidence, consistency of studies, risk-benefit ratio, patient preferences, ethical obligations and feasibility were also considered when grading recommendations (Table 2).

The Delphi process was implemented at two points throughout the CPG development process. The outline of the PICO questions and later the draft recommendation statements have been reviewed by an international panel of 16 experts, including a patient representative (KT, President of the European Federation of Associations for Patients with Haemochromatosis). The panel and the EASL Governing Board jointly nominated the group consulted by the Delphi process. The classification of consensus strength was as follows: Strong consensus if ≥95% agreement, consensus if ≥75-95% agreement, majority agreement if >50-75% agreement, no consensus if <50% agreement. The technical solution has been supported by the Clinical Guideline Service group (https://www.guideline-services.com), which has provided an online platform, where all CPG documents have been uploaded and reviewed.

Diagnosis

Who should be tested for haemochromatosis?

Recommendations

Individuals with clinical and biochemical signs of haemochromatosis, elevated transferrin saturation and high serum ferritin concentrations, or otherwise unexplained persistently elevated transferrin saturation should be genetically tested for haemochromatosis after informed consent for genetic testing has been obtained **(LoE 2, strong recommendation, strong consensus).**

Patients with increased liver iron evident on liver biopsy or MRI should be clinically assessed and biochemically tested for haemochromatosis (serum ferritin and transferrin saturation) **(LoE 2, strong recommendation, strong consensus).**

Adult individuals with a positive family history of first-degree relatives with haemochromatosis should be genetically tested for haemochromatosis after informed consent for genetic testing has been obtained **(LoE 4, strong recommendation, strong consensus).**

The clinical manifestations of haemochromatosis are dependent on disease stage and are determined by the degree of iron overload and the severity of organ damage. Screening studies have shown that at early stages, the disease is usually asymptomatic.[19] Common symptoms of haemochromatosis

Table 1. Level of evidence based on the Oxford Centre for Evidence-based Medicine (adapted from The Oxford 2011 Levels of Evidence[18]).

Level	Criteria	Simple model for high, intermediate and low evidence
1	Systematic reviews (SR) (with homogeneity) of randomised controlled trials (RCT)	Further research is unlikely to change our confidence in the estimate of benefit and risk
2	Randomised controlled trials (RCT) or observational studies with dramatic effects; systematic reviews (SR) of lower quality studies (*i.e.* non-randomised, retrospective)	
3	Systematic reviews (SR) of lower quality studies (*i.e.* non-randomised, retrospective)	Further research (if performed) is likely to have an impact on our confidence in the estimate of benefit and risk and may change the estimate
4	Case-series, case-control, or historically controlled studies (systematic review is generally better than an individual study)	
5	Expert opinion (mechanism-based reasoning)	Any estimate of effect is uncertain

Table 2. Grades of recommendation.

Grade	Wording	Criteria
Strong	Shall, should, is recommended. Shall not, should not, is not recommended	Evidence, consistency of studies, risk-benefit ratio, patient preferences, ethical obligations, feasibility
Weak or open	Can, may, is suggested. May not, is not suggested.	

include fatigue and joint pain.[8] In advanced cases, cardiac arrhythmia, impotence, and skin pigmentation are also present.[19] Patients with haemochromatosis are more commonly affected by liver disease including liver tumours, rheumatoid arthritis, osteoarthritis, osteoporosis, chondrocalcinosis and diabetes mellitus.[2]

As cardiomyopathy and hypogonadotrophic hypogonadism are more common in juvenile haemochromatosis, patients presenting with otherwise unexplained heart failure, abnormal sexual development in males or amenorrhea in females should also be tested for haemochromatosis.[4] (see Fig. 1)

Biochemical findings that indicate haemochromatosis with iron overload include elevated transferrin saturation, high ferritin and elevated liver transaminases.[20] From a clinical perspective, patients with any of the conditions listed above should be tested for haemochromatosis if transferrin saturation and ferritin are elevated.[21] Furthermore, serum ferritin and iron parameters should be determined as part of the work up for any individual with abnormal liver blood tests.[22]

Individuals with a positive first-degree family history of haemochromatosis should also be tested by *HFE* genotyping with appropriate genetic counselling because haemochromatosis is inherited in an autosomal recessive mode. The expected risk of a sibling of a patient with haemochromatosis being affected is 25%. A cost-effectiveness analysis has shown that, in families with ≥2 children, testing the index case's spouse before testing the children is cost effective.[23] Only adults should be tested for HFE-haemochromatosis, as the risk of disease penetrance increases with age.[24] For first-degree relatives of patients with non-HFE-associated haemochromatosis, screening should be done phenotypically if the specific genetic defect has not been identified or by targeted genotyping if the genetic defect is known.

What should be included in an iron panel to test patients for suspected haemochromatosis?

Recommendations

The first step in testing for haemochromatosis is the assessment of serum iron parameters, which should include transferrin saturation and serum ferritin **(LoE 3, strong recommendation, strong consensus).**

The concentration of serum iron and transferrin or total iron binding capacity can provide additional information for the differential diagnosis **(LoE 4, weak recommendation, consensus).**

Measuring hepcidin is not recommended **(LoE 5, strong recommendation, strong consensus).**

Haemochromatosis is associated with progressive iron overload, where elevated transferrin saturation is the first biochemical disease manifestation, which results from inappropriately low circulating hepcidin.[25] A transferrin saturation >50% in men and >45% in women has been defined as elevated.[8]

Transferrin saturation shows significant variability, which limits its usefulness. Blood samples for the measurement of transferrin saturation should be taken in the morning but fasting does not improve diagnostic utility.[26] Ferritin is not only a marker of iron overload, but also an acute phase reactant, tumour marker and indicator of increased angiogenesis. Ferritin is also released from necrotic or lysed cells. Serum ferritin concentrations are often elevated in conditions associated with fatty liver disease, such as excess alcohol consumption, or the metabolic syndrome.[27] These conditions must be considered when patients are investigated for high ferritin, as serum ferritin concentrations may be elevated in patients with co-existing

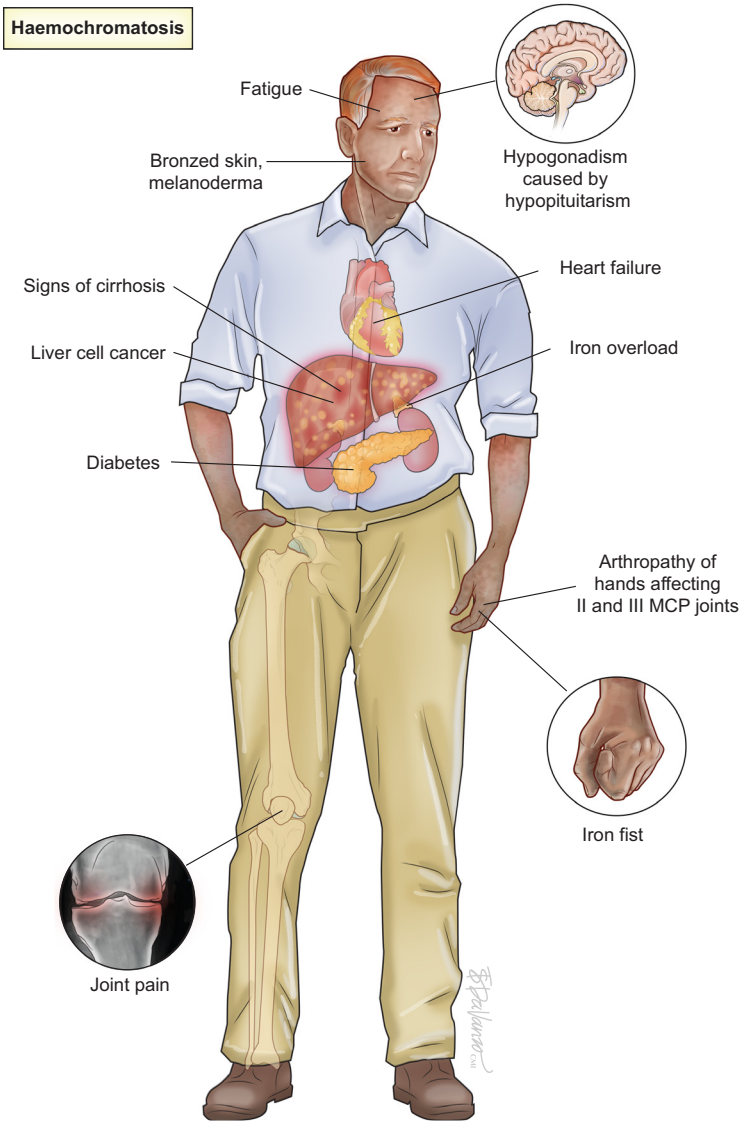

Fig. 1. Summary of the clinical manifestations of haemochromatosis. MCP, metacarpophalangeal.

haemochromatosis and fatty liver disease.[28] However, the presence of fatty liver does not exclude haemochromatosis.

The differential diagnosis of elevated transferrin saturation also includes conditions with decreased serum transferrin concentration such as alcohol-related liver disease or cirrhosis[29,30] and haematological diseases characterised by dyserythropoiesis, chronic haemolysis and/or transfusion regimen.[31] Chronic alcohol excess can increase serum ferritin concentrations in several ways by increasing iron absorption and liver injury.[32]

Although an inappropriately low hepcidin is implicated in the pathophysiology of iron overload in haemochromatosis, hepcidin has limited diagnostic utility, because reference ranges are assay-dependent and no universal reference ranges have been established.[33] Inappropriately high hepcidin can be an indicator of ferroportin disease.[34] Non-transferrin-bound iron is also frequently increased in patients with haemochromatosis but is not required for the diagnosis or differential diagnosis of haemochromatosis.[35]

Who should be tested for the p.C282Y variant in HFE?

Recommendations

Genotyping for p.C282Y in HFE should be carried out in individuals of European origin with biochemical evidence of iron overload (females with transferrin saturation >45% and serum ferritin >200 μg/L and males with transferrin saturation >50% and ferritin >300 μg/L, or otherwise unexplained persistently elevated transferrin saturation) with or without clinical signs or symptoms suggestive of haemochromatosis **(LoE 2, strong recommendation, strong consensus).**

Adult (>18 years of age) first-degree relatives of patients with p.C282Y homozygous haemochromatosis should be tested for the p.C282Y variant in HFE **(LoE 4, strong recommendation, strong consensus).**

Haemochromatosis is a clinico-pathological disorder characterised by biochemical evidence of altered plasma iron homeostasis, increased tissue iron deposition, and a genetically determined increased risk of cirrhosis, liver malignancy, osteoporosis or joint disease. The commonest disease-associated genotype is homozygosity for the p.C282Y variant in HFE, which is present in >80% of patients with clinically overt haemochromatosis.[36–38] The prevalence of the p.C282Y risk allele varies in Europe depending on the geographical region (Fig. 2). Although the reported penetrance is variable depending on the study and the population included, it is generally higher in men and increases with age. Haemochromatosis penetrance leading to end-organ damage in patients with p.C282Y homozygosity is only 50% in population-based studies.[39] This represents significant limitations for genotypic screening for haemochromatosis.[40,41]

In clinical practice, genotyping should follow phenotypic assessment of serum iron parameters and primarily be carried out in individuals with biochemical evidence of iron overload. There is no universally accepted threshold for ferritin or transferrin saturation indicating iron overload, but one seminal population screening study identified that transferrin saturation >45% and serum ferritin >200 μg/L for females and transferrin saturation >50% and ferritin >300 μg/L for males indicate iron overload.[8]

In a large cross-sectional study in a racially diverse primary health care population, 84% of men and 73% of women with homozygosity for the p.Cys282Tyr variant in HFE (p.C282Y) had an elevated transferrin saturation. A ferritin concentration >300 μg/L was present in 88% of p.C282Y homozygous males, and a ferritin concentration >200 μg/L was present in 57% of homozygous females.[21] Disease penetrance of HFE-haemochromatosis is not only determined by sex but also by age with increasing penetrance with older age.[24]

For the purposes of family screening, first-degree relatives of patients with genetically confirmed haemochromatosis should be genotyped, because the penetrance in family members of patients with haemochromatosis is higher than in the general population.[41] In clinical practice, genotyping should be combined with biochemical and phenotypic assessment for haemochromatosis, because homozygosity for p.C282Y alone is neither necessary nor sufficient for the diagnosis of haemochromatosis.[42] (Fig. 3)

Should patients also be tested for the p.H63D variant in HFE?

Recommendation

Genotyping for p.H63D can be performed in special clinical situations. In combination with p.C282Y and usually other acquired risk factors, p.H63D is associated with a higher risk of mild iron overload **(LoE 5, weak recommendation, n.a.).**

Statement

Genotyping for p.H63D is not generally suggested to guide treatment, but the value of genotyping for this variant remains controversial **(n.a.).**

The prevalence of p.H63D in the general population is high, indicating that it may be a benign polymorphic variant.[43] Compound heterozygosity for p.C282Y and p.H63D alone appears

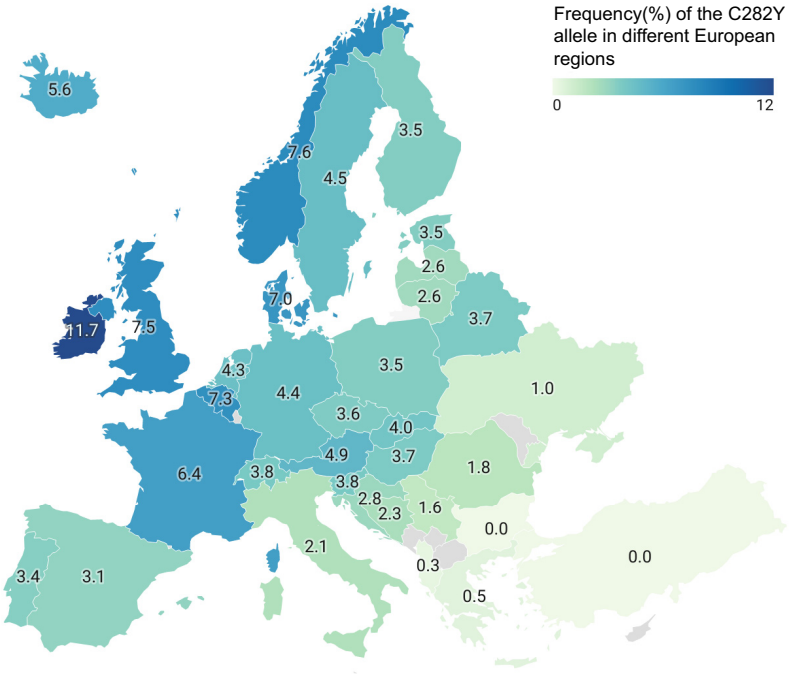

Fig. 2. Prevalence of the p.C282Y risk allele for haemochromatosis in Europe. Note that allele frequency is subject to significant variation in specific geographical regions. One example for such variation is Portugal, where the risk allele prevalence is 5.8% in the North compared to 0.9% in the South.[183]

insufficient to cause haemochromatosis, but when this genotype coincides with additional genetic or environmental risk factors for the development of liver disease, affected individuals can present with phenotypic haemochromatosis.[44] Homozygosity for p.H63D is not more common among patients with non-p.C282Y homozygous haemochromatosis than in the general population.[37]

The clinical value of testing for p.H63D remains controversial. When *HFE* was first identified as the haemochromatosis gene, p.C282Y and p.H63D were identified as disease-associated variants. In that study, the pooled prevalence of compound heterozygosity for p.C282Y/p.H63D among patients with clinically characterised haemochromatosis was 4.1% (114/2,802), which was higher than 1.6% (100/6,243) in control populations.[37]

In the eMERGE cohort comprising 39,000 individuals, the diagnostic rate of compound heterozygosity for p.C282Y/p.H63D was significantly lower than that of homozygosity for p.C282Y (2.3% *vs*. 14% in females and 3.5% *vs*. 24.4% in males, respectively).[24] In patients with iron overload and compound heterozygosity in HFE, the coincidence of disease-modifying factors such as diabetes, fatty liver, obesity or alcohol consumption is higher than in patients with homozygosity for p.C282Y.[45,46] In a large prospective population-based study of 31,192 individuals of northern European descent, it was shown that serum iron indices in p.C282Y/p.H63D compound heterozygotes do not change during middle life in males, and that in females, despite increasing ferritin levels with age, iron overload-related disease is rare.[47] In a large UK community sample of volunteers of European descent aged 40 to 70 over a mean follow-up of 7 years, incident haemochromatosis diagnoses were more common in p.C282Y/p.H63D heterozygous individuals (hazard ratio [HR] 33.63; 95% CI 21.44-52.76; p <0.001 for men; HR: 34.74; 95% CI 16.47-73.29; p <0.001 for women) compared to participants with neither p.C282Y nor p.H63D variants, although the excess morbidity was no longer significant after correction for multiple testing.[2] The question of making a diagnosis of haemochromatosis in p.C282Y/p.H63D compound heterozygotes with mild iron overload as opposed to considering them individuals at risk for acquired iron overload was addressed in the latest guidelines on the molecular diagnosis of haemochromatosis.[2] A meta-analysis supports that this genotype is considered insufficient to cause haemochromatosis, although p.C282Y/p.H63D compound heterozygosity is a risk factor for slightly increased serum iron parameters and mildly increased hepatic iron stores.[48]

It is very rare for compound heterozygosity for p.C282Y/p.H63D to be associated with a severe iron overload phenotype in the absence of acquired causes; hence, in such cases, a search for other non-HFE-haemochromatosis gene mutations is warranted.[46] The p.H63D genotype might be considered a genetic variant that predisposes to slight alterations in iron parameters but not a disease-causing variant; therefore, special care is recommended when reporting and interpreting its presence in a genetic test for haemochromatosis.[6]

Members of the expert panel agree that testing for the p.H63D variant is not necessary for the diagnosis of haemochromatosis. If the testing strategy includes *HFE* genotyping and patients with iron overload and p.C282Y/p.H63D compound heterozygosity or homozygosity for p.H63D are identified, they should be investigated for additional environmental or additional genetic risk factors to explain the iron overload phenotype. As patient associations struggle with the confusing nomenclature in the literature, they ask for clear expert recommendations to respond with confidence to the questions raised by patients carrying the p.H63D variant.

In clinical practice, most genetic laboratories report p.C282Y and p.H63D simultaneously in the same test, making it difficult to change this practice. Compound p.C282Y/p.H63D heterozygotes with normal serum iron test panels and without evidence of iron overload can be advised to monitor iron parameters routinely. Other arguments that support abandoning p.H63D genotyping for haemochromatosis include the risk of promoting incorrect interpretation of genotype and consequently incorrect management and treatment decisions in patients with iron overload who are not homozygous for p.C282Y. Finally, the routine practice of testing for the p.H63D variant may perpetuate the current confusion regarding the use of *HFE* genotyping either as a diagnostic tool or as a genetic susceptibility test.[6]

How should patients with p.C282Y/p.H63D compound heterozygosity or p.H63D homozygosity be managed?

Recommendations

The management of patients with p.C282Y/p.H63D compound heterozygosity or p.H63D homozygosity should be guided by their phenotypic presentation and the presence of additional risk factors, not the genotype alone **(LoE 5, strong recommendation, strong consensus).**

Patients who are compound heterozygous for p.C282Y/p.H63D or homozygous for p.H63D with confirmed iron overload should be investigated for other causes of iron overload **(LoE 4, strong recommendation, consensus).**

Patients who are compound heterozygous for p.C282Y/p.H63D or homozygous for p.H63D with confirmed iron overload (by MRI or liver biopsy) may be treated with phlebotomy, but this treatment decision requires individualised clinical assessment **(LoE 5, weak recommendation, strong consensus).**

The management of patients with iron overload who are not p.C282Y homozygotes is determined by their phenotypic presentation and the presence of associated risk factors. Although many patients are referred for phlebotomy (therapeutic venesection) in the setting of a risk genotype, the benefits are largely unclear.

When p.C282Y/p.H63D compound heterozygosity is detected by a predictive test in asymptomatic individuals who do not show evidence of iron overload, the risk of them developing significant iron overload is low.[47,49] Because they may be at risk of developing mild iron overload in association with additional environmental risk factors, it is important that they are instructed to maintain a healthy lifestyle.[50] Depending on clinical judgment, compound heterozygotes without iron overload can be advised to monitor serum iron parameters, where monitoring intervals can be determined by age and risk profile. It is unclear if p.C282Y/p.H63D compound heterozygous or p.H63D homozygous patients with

mild signs of iron overload (increased serum ferritin) benefit from phlebotomy.

The management of patients with iron overload who are not p.C282Y homozygotes has not been addressed in specific studies. Management of additional environmental risk factors or associated liver diseases is crucial. Iron overload should be managed by phlebotomy and it has been reported that between 14% and 30% of patients referred for phlebotomy are compound heterozygotes.[51] Iron overload is rare in individuals homozygous for p.H63D and was present in only 3.2% in one study.[52] Another study showed that although transferrin saturation is elevated in patients with p.H63D homozygosity, high ferritin is rare in these patients.[30] In patients with excessive alcohol consumption and elevated serum iron parameters, the risk of fibrosis and hepatocellular malignancy is increased.[53] Weight loss through dietary modification along with increased physical activity should be recommended in patients with fatty liver disease.[54]

In whom should non-invasive tests for quantification of iron overload be performed?

Recommendations

In patients with an unclear cause of hyperferritinemia, biochemical iron overload (increased transferrin saturation and ferritin) or positive liver iron staining, MRI should be used to quantify hepatic iron concentrations and to assess extrahepatic organ involvement **(LoE 4, strong recommendation, strong consensus).**

Cardiac MRI can be performed in patients with haemochromatosis and signs of heart disease, and in juvenile forms of haemochromatosis **(LoE 5, strong recommendation, strong consensus).**

Statement

MRI is helpful for detection, non-invasive quantification of iron, and to study the distribution of iron in the liver, spleen, pancreas, heart, and brain in patients with suspicion or diagnosis of iron overload disorder. In patients with suspected aceruloplasminemia, MRI of the brain can also provide important additional information **(strong consensus).**

Serum iron parameters are surrogates of body iron status. Elevated transferrin saturation, which is characteristic of haemochromatosis can result from inappropriately low hepcidin concentration and indicates disturbed plasma iron homeostasis.[25] Increased alcohol consumption is an alternative cause of elevated transferrin saturation.[53,55–57] Elevated ferritin can indicate increased tissue iron concentrations but is also non-specific. Alternative causes include inflammatory and neoplastic conditions.[28,56] Therefore, serum iron parameters alone are often insufficient to ascertain iron overload. Tissue iron concentrations can be assessed non-invasively by MRI, where specific relaxation sequences have been adopted to reliably quantify iron. R2* sequences are the best validated.[58,59]

MRI can be associated with high cost and limited accessibility, however validated software for use in hepatic iron quantification is freely available.[60,61] In patients homozygous for the p.C282Y variant of the *HFE* gene, with elevated transferrin saturation and hyperferritinemia without additional risk factors, non-invasive assessment of tissue iron overload by MRI is not required for the diagnosis, but enables determination of the degree of iron overload, which is a predictor of organ damage. Hepatic MRI R2* quantification can act as a surrogate of total body iron stores (as measured by quantitative phlebotomies[58]); therefore, MRI is also a predictor of the number of phlebotomies required for intensive treatment.[58]

In patients without homozygosity for p.C282Y and/or the presence of additional risk factors for hepatic iron overload, such as the metabolic syndrome and chronic alcohol excess, non-invasive quantification of liver, spleen, pancreas and cardiac iron can guide diagnosis and management. In patients with metabolic syndrome and, more often, with increased alcohol consumption, as well as in patients with cirrhosis or chronic liver disease, iron overload may also be present.[62] However, there is no conclusive evidence to support the use of iron depletion through phlebotomy in these patients. At early disease stages, haemochromatosis and aceruloplasminemia are associated with predominant hepatic and no or minimal spleen iron overload. In contrast, spleen iron overload is increased in ferroportin disease and transfusional iron overload.[61,63]

When is a liver biopsy required in patients with haemochromatosis?

Recommendations

Liver biopsy aims to assess the presence of cirrhosis; therefore, in patients with an otherwise clear diagnosis of cirrhosis, liver biopsy is not recommended **(LoE 5, weak recommendation, consensus).**

Liver biopsy can be performed to assess liver fibrosis if serum ferritin is higher than 1,000 μg/L or if liver enzymes are increased **(LoE 4, strong recommendation, strong consensus).**

Liver biopsy is not recommended for the diagnosis of hepatic iron overload **(LoE 5, weak recommendation, consensus).**

With the development of non-invasive methods to quantify iron burden, liver biopsy is now rarely performed to assess the severity of liver fibrosis or degree of iron overload. In patients with haemochromatosis, liver iron overload induces liver fibrosis, and severe liver fibrosis is associated with an increased risk of HCC.[64] Definitive mechanisms remain unclear but are likely to involve the activation of hepatic stellate cells, hepatocyte senescence, and reactive oxygen species generated by excess tissue iron.[65,66] Accordingly, the degree of liver fibrosis correlates

with the severity of iron overload. Although the level of iron burden that is associated with cirrhosis is not clear and varies according to the presence of other factors, large scale studies have demonstrated that if serum ferritin is lower than 1,000 μg/L the risk of severe liver fibrosis is negligible.[67,68] A serum ferritin <1,000 μg/L, in the absence of hepatomegaly, thrombocytopenia, and abnormal transaminases was associated with a negative predictive value of 94% for advanced liver fibrosis.[67,69] In the presence of other chronic liver diseases, this might be less clear; therefore, if serum ferritin is lower than 1,000 μg/L but transaminases are increased, a liver biopsy may be considered. However, the 1,000 μg/L cut-off may be a conservative approach, considering the advent of non-invasive scores and tests (although not validated in patients with haemochromatosis); for this reason, liver biopsy is less and less frequently performed. Nonetheless, there is currently insufficient evidence to recommend a specific ferritin cut-off at which liver biopsy must be performed. Thus, in patients with serum ferritin higher than 1,000 μg/L, liver biopsy can be considered, while taking into account the iron burden, other cofactors for liver disease, and the interpretation of non-invasive tests. In patients with haemochromatosis in whom cirrhosis could neither be ruled out nor be confirmed by other means, liver biopsy should be performed.

How and when should fibrosis stage be assessed?

Recommendations

All patients with haemochromatosis should be non-invasively assessed for the presence of liver fibrosis at diagnosis to guide appropriate treatment and follow-up **(LoE 4, strong recommendation, strong consensus).**

Transient elastography can be used to rule out advanced fibrosis in patients with haemochromatosis if liver stiffness is ≤6.4 kPa **(LoE 4, weak recommendation, consensus).**

Statements

FIB-4 is the serum-based non-invasive marker which is best evaluated for the assessment of fibrosis stage in patients with chronic liver diseases, but the available evidence is limited in patients with haemochromatosis **(strong consensus).**

In patients with ferritin <1,000 μg/L, normal transaminases and no liver enlargement, the risk of advanced liver fibrosis is very low **(consensus).**

Non-invasive scores such as APRI (aspartate aminotransferase-to-platelet ratio index) and FIB-4 (patient age, platelet count, aspartate aminotransferase and alanine aminotransferase) are simple to use and have been evaluated for the detection of advanced fibrosis in haemochromatosis, but reported thresholds were lower than in other chronic liver diseases.[70] A small single-centre study using transient elastography in haemochromatosis reported a high negative predictive value for excluding advanced liver fibrosis, but larger studies are required.[71,72]

Liver biopsy is reserved for patients in whom cirrhosis can neither be ruled out nor confirmed (see above and Fig. 4), because the decision of when to start surveillance for HCC depends on a diagnosis of cirrhosis.[73]

Hepatic iron overload and associated tissue damage is reflected by the degree of liver fibrosis.[71,74] Fibrosis indicates progressive liver disease.[75] Few studies have validated diagnostic test performance of serum-based fibrosis tests and elastography methods in haemochromatosis, but available data indicate that the threshold for the diagnosis of advanced fibrosis may be lower in haemochromatosis than in chronic viral hepatitis, alcohol-related liver disease and metabolic-associated fatty liver disease.[76] Fibrosis assessment by magnetic resonance elastography is complex because iron overload interacts with the magnetic resonance signal, rendering fibrosis assessment difficult in patients with hepatic iron overload.[77] Patients with haemochromatosis have a distinct pattern and mode of fibrosis progression, which is different from chronic viral hepatitis or alcohol-related liver disease.[78] No evidence-based recommendations on thresholds for the detection of advanced fibrosis, for follow-up intervals, nor for management can currently be provided; hence, future studies are needed.

Which extrahepatic manifestations should be investigated?

Recommendations

Clinicians should clinically evaluate patients with haemochromatosis for extrahepatic manifestations including skeletal (joint pain, arthritis, osteoporosis, fractures) and endocrine (diabetes) manifestations, and reproductive or sexual dysfunction (erectile dysfunction, loss of libido or amenorrhea) **(LoE 3, strong recommendation, strong consensus).**

Patients with severe iron overload should be evaluated for arrhythmia and cardiac dysfunction (electrocardiogram [ECG] and echocardiography) **(LoE 4, strong recommendation, strong consensus).**

Patients with severe haemochromatosis and signs or symptoms of heart disease (conduction disease and/or contractile dysfunction) should be investigated with cardiac MRI for iron quantification without delaying treatment **(LoE 4, strong recommendation, strong consensus).**

Patients with juvenile haemochromatosis should be investigated for cardiac involvement, including myocardial iron quantification by MRI **(LoE 4, strong recommendation, strong consensus).**

Joint disease and osteoporosis are common in patients with haemochromatosis and do not respond uniformly to phlebotomy; hence, they merit investigation and specific treatment where appropriate. A recent survey of patients with haemochromatosis demonstrated that extrahepatic manifestations are common. Of 1,689 respondents, 86.5% described arthritis or joint pain, 81.3% fatigue, 73.1% psychological or cognitive difficulties

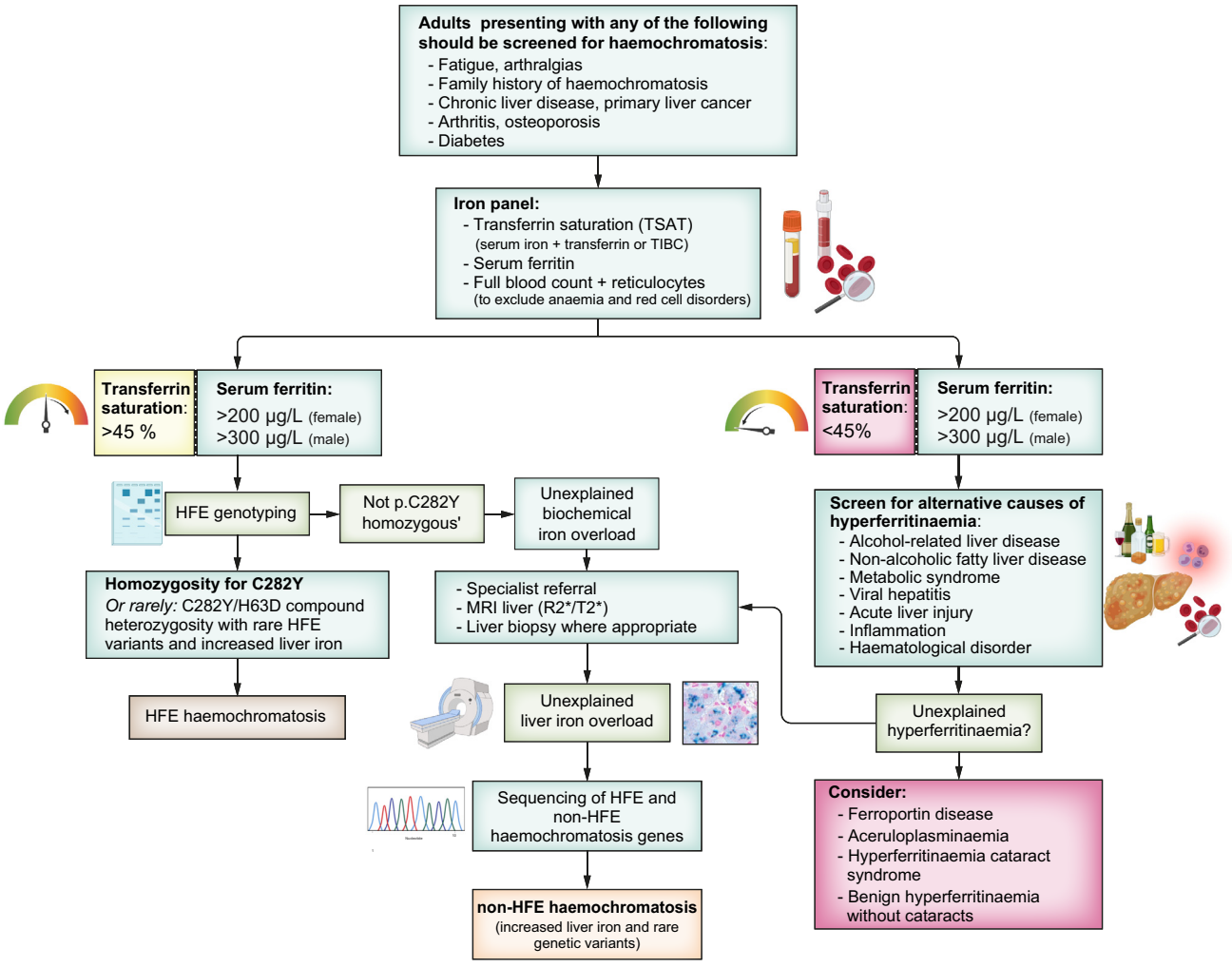

Fig. 3. Algorithm for the diagnostic approach to patients with hyperferritinemia and suspected haemochromatosis. In patients of non-European origin with clinical suspicion of haemochromatosis and elevated transferrin saturation and ferritin, the pre-test likelihood for detecting the p.Cys282Tyr variant in HFE is very low. Therefore, in individuals of non-European origin, direct sequencing of HFE and non-HFE genes may be considered without HFE genotyping. Non-HFE gene sequencing should encompass a panel of genes including *HFE, HJV, TFR2, CP* and *SLC40A1*. TIBC, total iron binding capacity.

(38% depression, 60.4% brain fog), 70.4% skin problems, 62.9% of females menstrual problems, and 57.3% sexual health issues.[79] Of those describing musculoskeletal symptoms, the predominant joints affected were ankles (69.3%), hip (56.8%) and hand/wrist (46.9%).

There is an increased prevalence of joint disease in haemochromatosis. A population study of 2,890 p.C282Y homozygotes aged 40 to 70 showed that this population had increased rates of osteoarthritis and hip replacement compared with people with no p.C282Y mutations.[2] In a study of 3,531 patients, non-infectious arthropathies were more frequent with a HR of 2.38 (95% CI 2.14-2.64). Patients were also at increased risk of hip replacement (HR 2.77, 95% CI 2.27-3.38) and knee replacement (HR 2.14, 95% CI 1.58-2.88) surgery. A further study showed that p.C282Y homozygotes had an increased risk of single total hip replacement (HR 1.94, 95% CI 1.04-3.62) and bilateral total hip replacement (odds ratio 5.86, 95% CI 2.36-14.57) for osteoarthritis.[80]

The joint disease in haemochromatosis is similar to osteoarthritis but is associated with a younger age of onset; haemochromatosis arthropathy typically affects the 2nd and 3rd metacarpophalangeal joints and ankles, and is characterised by exuberant osteophytes and rapid progression to cartilage loss.[81] Radiographs show degenerative changes with joint space narrowing, osteophytes, and subchondral cysts, with chondrocalcinosis present 50% of the time. Iron deposition in joints is not universally observed.[82] The histological picture of the synovium in haemochromatosis arthropathy largely resembles a process reminiscent of osteoarthritis. Neutrophil invasion is, however, markedly increased in haemochromatosis arthropathy, especially in joints with iron deposition, which may accelerate cartilage degradation.[3,83]

Haemochromatosis arthropathy does not respond to phlebotomy and can develop on maintenance therapy. Symptoms can significantly affect quality of life. Treatment is limited to analgesics and non-steroidal anti-inflammatory agents, physiotherapy and ultimately orthopaedic procedures including joint replacement.[84]

In severe haemochromatosis (mostly in juvenile forms and rarely in adult forms with severe or advanced disease), endocrine and cardiac manifestations may be apparent.

Cardiomyopathy and endocrine failure (mainly pituitary hypogonadism) are particularly prevalent clinical manifestations

in HJV and HAMP-related haemochromatosis,[85,86] as recently confirmed in two systematic reviews of 156 patients with non-HFE-haemochromatosis (including 99 HJV and 11 HAMP-related forms)[87] and 132 HJV-haemochromatosis cases.[88]

If not treated, affected patients can develop severe heart failure before age 30, possibly resulting in death or the need for a heart transplant.[85,86,88] Autopsy and endomyocardial biopsy studies showed that iron deposition is sarcoplasmic and not interstitial, suggesting a storage disease rather than an infiltrative process.[89]

Myocardial iron accumulation induces the development of restrictive cardiomyopathy with early diastolic dysfunction that may progress towards dilated cardiomyopathy with impaired systolic function.[90] Myocardial iron overload often precedes cardiac dysfunction and ventricular diastolic dysfunction can be present in asymptomatic patients.[86] Deposition of iron may also involve the conduction system, especially the atrioventricular node. A variety of conduction disturbances have been described in patients with iron overload cardiomyopathy, including extreme bradycardia, tachycardia, supraventricular (mainly atrial fibrillation) and ventricular arrhythmias, any degree atrioventricular blocks, and sudden death,[86,90–92] some of which are found in clinical practice in patients with severe juvenile haemochromatosis even in the absence of congestive heart failure (*e.g.* extreme bradycardia and heart blocks often necessitating pacemaker implantation). It is worth noting that most of the data on iron-related heart disease are derived from studies on iron-loading anaemias and transfusional iron overload, where additional factors (*e.g.* chronic anaemia, chronic haemolysis, endothelial and vascular dysfunction) can affect cardiac pathology.

Clinical investigation includes evaluation of signs and symptoms of contractile dysfunction or conduction disease, ECG, Holter-ECG, and transthoracic echocardiography and is best completed using MRI R2* to assess the degree of myocardial iron overload.[93] Although developed and validated in patients with thalassemia syndromes, myocardial MRI R2* can help stratify patient risk, determine the need for an intensive iron removal strategy and optimise follow-up.[90,91,94]

There are no recognised threshold levels for serum ferritin or liver iron concentration associated with the presence of cardiac iron deposition or with the need for cardiologic referral in patients with haemochromatosis. Data from historical series of patients with HFE and non-HFE-haemochromatosis, systematic reviews,[87] clinical practice, and iron biology and toxicity studies[31] indicate that the rate and magnitude of the circulatory and tissue iron overload determine the haemochromatosis phenotype and that the heart is particularly sensitive to a rapid and massive increase in plasma iron early in disease development.

Therefore, in patients with HFE-haemochromatosis, a structured patient interview, physical examination and cardiac investigations should be reasonably guided by the overall assessment of age at diagnosis, clinical manifestations, and cofactors of iron overload. A similar approach can be applied to TFR2-related haemochromatosis, albeit with a lower threshold for initiating cardiac investigations because of its intermediate phenotype (regarding onset and clinical manifestations) – between HFE- and HJV-related haemochromatosis. A conservative approach with echocardiography and/or cardiac MRI is advisable.[85,87]

Conversely, due to the high prevalence of cardiac involvement in juvenile forms, all patients should be thoroughly investigated at the time of diagnosis, and follow-up should be adapted to the severity and stage of the disease, clinical manifestations, effectiveness of iron removal, and patient's adherence to therapy.

Cardiologist consultation is recommended in the presence of signs of possible cardiac involvement. In patients with heart failure and arrhythmias, conventional treatment should be initiated per standard cardiology practice. Iron removal therapy can prevent, improve, or even reverse cardiac dysfunction.[90] Endocrine investigations should also be guided by clinical symptoms and include assessment for diabetes, measurement of sex hormone concentrations, and rarely thyroid, adrenal as well as parathyroid status.

Which patients should undergo screening for HCC and how often?

Recommendations

Patients with haemochromatosis and cirrhosis (on biopsy METAVIR F4; Ishak scoring system stage 6 or on elastography) should undergo HCC screening every 6 months, regardless of iron depletion **(LoE 4, strong recommendation, strong consensus).**

HCC screening every 6 months can be suggested in patients with haemochromatosis and advanced fibrosis (*i.e.* bridging fibrosis; METAVIR F3; Ishak scoring system stages 4-5), regardless of iron depletion **(LoE 4, weak recommendation, consensus).**

Patients with haemochromatosis and regression of advanced fibrosis or cirrhosis to a stage of F2 or less after treatment should continue HCC screening, but the surveillance interval may be individualised **(LoE 4, strong recommendation, strong consensus).**

Surveillance of HCC should be performed by experienced personnel using abdominal ultrasound every 6 months **(LoE 5, strong recommendation, consensus).**

When ultrasound evaluation is technically suboptimal, HCC surveillance should be performed by MRI or CT **(LoE 5, strong recommendation, consensus).**

Imaging-based surveillance of HCC can be facultatively performed in combination with serum alpha-fetoprotein (AFP) every 6 months **(LoE 5, weak recommendation, consensus).**

Statement

HCC risk in patients with haemochromatosis and pre-treatment advanced fibrosis is modified by the presence of additional risk factors for liver cancer such as alcohol consumption, type 2 diabetes mellitus, and viral hepatitis, but validated risk scores for HCC are not available to tailor surveillance in patients with haemochromatosis **(consensus).**

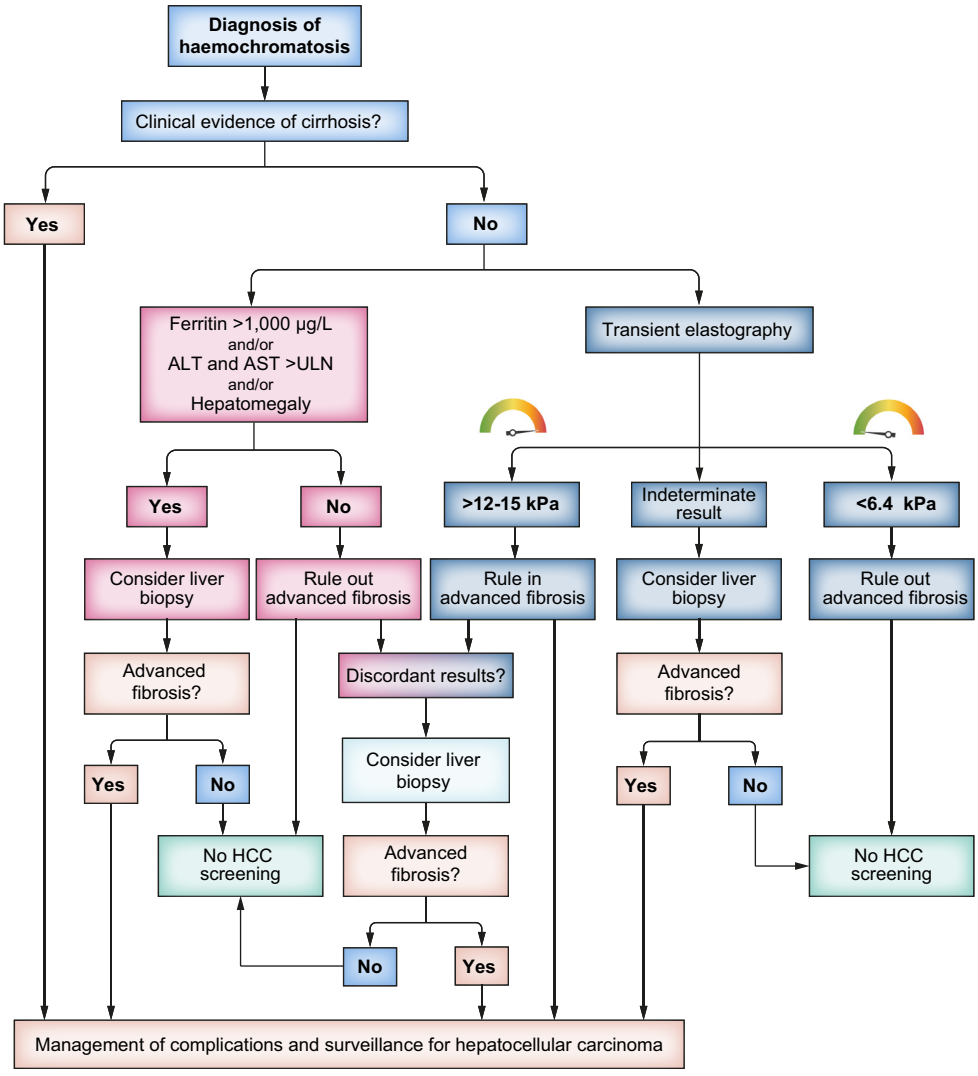

Fig. 4. Recommendations for fibrosis staging in patients with haemochromatosis. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal.

Cirrhosis and HCC are known complications of haemochromatosis whose frequency is controversial, depending on the type of study (population- or patient-based studies, cross-sectional studies or estimates of lifetime cumulative penetrance of disease).[73,95,96] In haemochromatosis cohorts, a relative risk of primary liver cancer of between 20 and 200 was calculated,[97–99] including descriptions of biliary differentiation (cholangiocarcinoma or hepato-cholangiocarcinoma) in up to 35% of cases from liver cancer series.[64,98,100] More recently, in the large UK Biobank population cohort, p.C282Y homozygote males aged 40 to 70 had a higher incidence of any liver disease (HR 2.34, 95% CI 1.60-3.43, p <0.001) and liver cancer (HR 8.88, 95% CI 4.79-16.45, p <0.001) compared to individuals without the p.C282Y or the p.H63D variant, with male sex increasing the estimated effect size.[2] These data were confirmed in a subsequent analysis on the same community cohort, showing that male p.C282Y homozygotes had a significantly increased risk of incident primary hepatic malignancy (including hepatocellular and intrahepatic bile duct carcinomas) (HR 10.5, 95% CI 6.6-16.7, p <0.001) and all-cause death (HR 1.2, 95% CI 1.0-1.5, p = 0.046), compared to individuals without p.C282Y or p.H63D variants. Moreover, in lifetable projections for p.C282Y homozygous male individuals up to age 75 years, the risk of hepatic malignancies was 7.2% (95% CI 3.9%-13.1%) and the risk of death was 19.5% (95% CI 15.8%-24.0%), compared with 0.6% (95% CI 0.4%-0.7%) and 15.1% (95% CI 14.7%-15.5%), respectively, for men with neither variant. Among females, the association between p.C282Y homozygosity and liver cancer or death was not statistically significant.[1] In a recent large multicentre cohort study collecting retrospective data from 8 Swedish university hospitals, an increased risk of HCC was found in patients with p.C282Y homozygosity or p.C282Y/H63D compound heterozygosity (HR 21.32, 95% CI 10.34-43.97), although most HCC developed in p.C282Y homozygotes. The risk of all-cause mortality was also increased in patients with either pC282Y homozygosity or p.C282Y/H63D compound heterozygosity (HR 1.16, 95% CI 1.04-1.30), in comparison to population-based reference individuals, matched by age, sex, and county of residence.[5]

Cost-effectiveness studies indicate that an HCC incidence of at least 1.5% per year is required for HCC screening to increase longevity in patients with cirrhosis, independently of aetiology of liver disease.[73,101] Although the exact annual incidence of HCC

in haemochromatosis remains undefined, it has been estimated to be close to or above 1.5%.[101,102] The overall prevalence of HCC in patients diagnosed with haemochromatosis is approximately 10-30%, almost exclusively occurring in patients with cirrhosis[98,99,103–106] and independently of iron depletion.[98,100,107] Occurrence of HCC has also been described in non-cirrhotic stages in small series of patients with advanced fibrosis[13,108,109] and in sparse case reports of patients with moderate, mild or even absent fibrosis.[106,110,111] In their CPGs on HCC management, the European Association for the Study of the Liver (EASL) suggest that non-cirrhotic F3 patients, regardless of aetiology, may be considered for surveillance based on an individual risk assessment (evidence low, recommendation weak).[73] This recommendation is based on the fact that patients with chronic hepatitis C and bridging fibrosis have been shown to be at risk of HCC,[112] possibly related to under-staging of liver biopsy, rapid worsening of liver disease stage, and difficulties defining the transition from advanced fibrosis to cirrhosis. Stages 4 and 5 of the Ishak system, representing advanced bridging fibrosis and/or early nodule formation, have shown excellent correlation with F3 in the METAVIR system.[113] Based on the above premises, although information about the HCC incidence in patients with non-viral liver disease without cirrhosis is limited,[73] it seems reasonable to recommend HCC surveillance for patients with haemochromatosis and bridging fibrosis, especially in the presence of adjunctive risk factors for cancer. In fact, risk factors for HCC in patients with haemochromatosis have been described: higher age at disease diagnosis, high iron overload, duration of exposure to iron excess, male sex, presence of diabetes, and co-carcinogenic factors, such as alcohol intake, tobacco smoking, and HBV and HCV infections.[13,98,106,108,114] Also, other risk factors for cancer development, such as non-alcoholic fatty liver disease, obesity, exposure to radiation, chemicals, drugs and toxins, gene variants, and family history of HCC could play a role in some patients. Thus, specific individual information could be used to refine patient-tailored liver cancer surveillance, based on physician's judgment. Further studies could help formulate more accurate recommendations for liver cancer surveillance in patients with haemochromatosis in cirrhotic and non-cirrhotic stages. At present, clinicians should have a low threshold for ordering ultrasound when symptoms and/or biochemical/clinical evidence of deteriorating liver function appear.

As early as 50 years ago, at least partial regression of cirrhosis after iron depletion had been documented in case reports and series of patients with a clinical diagnosis of haemochromatosis.[115–118] Subsequent larger studies in genetically confirmed haemochromatosis corroborated the reversibility of hepatic fibrosis after phlebotomy, showing a significant improvement of fibrosis stage in patients without cirrhosis,[98,119] and even the regression of bridging fibrosis and initial cirrhosis in 69% and 35% of patients, respectively.[120] More recently, a retrospective study on a large population of patients with HFE-haemochromatosis, with sequential liver biopsies and long-term follow-up, was conducted to assess the regression of severe fibrosis and the risk of liver cancer after treatment.[13] Regression of fibrosis stage was recorded in approximately 70% of F3 patients and 20% of F4 patients after a median of 9.5 years between the first and the last biopsy. Of note, in patients with biopsy-proven fibrosis regression to a stage of F2 or less, the long-term risk for liver cancer was significantly reduced over a median follow-up of 19.1 years.[13] Further research is needed on the optimal timing and methods for assessing fibrosis regression in iron-depleted patients, and on liver cancer surveillance in patients who reach a post-treatment stage of F2 or less. At present, since the regression of advanced fibrosis and cirrhosis does not imply the regression of molecular changes involved in liver carcinogenesis and the full reversion of hepatic microvascular and micro-architectural changes, surveillance for liver cancer should be continued in patients with haemochromatosis and pretreatment cirrhosis or advanced fibrosis.

The poor prognosis of HCC is largely related to late diagnosis.[121] Even in the absence of randomised controlled trials in patients with cirrhosis, the overall data suggest that HCC surveillance is associated with significant improvement in early tumour detection, curative treatment rates, and survival.[122] Regardless of the cause of liver disease, screening to detect an early tumour by ultrasound examination at 6-month intervals is strongly recommended by EASL[73] guidelines. Serum AFP accuracy has mostly been tested for HCC diagnosis rather than surveillance,[73] and its sensitivity and specificity vary widely among studies.[123] Although the performance of AFP measurement alone as a test for routine HCC surveillance is suboptimal,[73] systematic meta-analysis have shown that adding AFP to ultrasound increases detection rates of either early or any stage HCC in patients with cirrhosis, while increasing false-positive rates.[123,124] On this basis, AFP can be added to ultrasound for surveillance of HCC in patients with haemochromatosis, although it is considered optional in recent European and American guidelines.[73,101] When ultrasound evaluation of the liver is technically suboptimal (*e.g.* severe parenchymal heterogeneity, severe obesity, hepatic steatosis), other modalities such as CT or MRI with contrast may be appropriate, instead of or in conjunction with ultrasound.[73,101,121,123] Finally, since its goal is to improve patient survival, HCC screening should be carried out only in individuals eligible for cancer treatment or liver transplantation.[73,101] Accordingly, HCC surveillance is recommended in patients with haemochromatosis and cirrhosis in Child-Pugh stage A and B, and in Child-Pugh stage C awaiting liver transplantation.[73,101]

When should rare haemochromatosis gene variants be tested?

Recommendations

Young individuals with biochemical evidence and clinical manifestations of haemochromatosis (liver disease, amenorrhea, hypogonadism, cardiomyopathy) should be tested for rare haemochromatosis gene variants **(LoE 4, strong recommendation, strong consensus).**

Patients with evidence of significant, unexplained iron overload should be referred for assessment by a specialist in iron disorders **(LoE 5, strong recommendation, strong consensus).**

Adult first-degree relatives of patients with rare variants in haemochromatosis genes should be tested for these variants; particular focus should be given to siblings as they are at the highest risk of haemochromatosis **(LoE 5, strong recommendation, strong consensus).**

According to the phenotypic definition, HFE p.C282Y homozygosity is found in 80% to 95% of patients with haemochromatosis.[7,36,128] Because of its high prevalence, simplicity, low cost and wide availability, genotyping for the p.C282Y variant in *HFE* is the first genetic test to be performed in patients with haemochromatosis. However, this might be questioned in populations with lower prevalence.[66]

In patients with haemochromatosis who are not homozygous for p.C282Y, testing for rare variants is suggested to provide a definite diagnosis in order:

i) to reassure patients by reducing uncertainty
ii) to improve disease prognostication and trigger organ-specific tests (*e.g.* ECG and echocardiography in TFR2 haemochromatosis)
iii) to allow for family screening
iv) to better understand disorders of iron metabolism

As our understanding of iron metabolism has improved, several genetic defects that are associated with haemochromatosis have been identified. The minimum gene-set for the assessment of haemochromatosis should at least include the following genes: *HFE, HAMP, HJV, TFR2, TF, CP, BMP6, SCL40A1*.[63] If available, clinical exome sequencing or panel gene sequencing can be used to cover even more candidate genes after appropriate informed consent has been obtained, but the interpretation of results may be challenging.[10]

The phenotype associated with these genes is variable and the appropriate tests should be discussed with specialists in disorders of iron metabolism. This discussion will also seek to balance the phenotype and family history, with comorbidities, environmental, nutritional and lifestyle factors, which co-determine the risk of iron overload. There is no available data to provide clear cut-off values that should trigger genetic testing.

With the advent of next-generation sequencing, large panels of genes are usually studied. Results are sometimes difficult to interpret and discussions with an experienced team are useful to assess the causality/pathogenicity of variants.[129]

As discussed, HFE compound p.C282Y/p.H63D and p.H63D homozygosity are not sufficient to induce significant iron overload without additional factors; thus, rare haemochromatosis gene variants should be tested in patients with these genotypes and confirmed severe iron overload, in the absence of other obvious causes of excess iron.

Management

Pregnancy

Recommendations

In patients with haemochromatosis planning to get pregnant, iron deficiency should be avoided **(LoE 4, strong recommendation, strong consensus).**

In pregnant women with mild to moderate iron overload without signs of advanced liver disease, decisions regarding therapeutic phlebotomy can be individualised but phlebotomy can be paused for the duration of pregnancy in most patients **(LoE 5, weak recommendation, n.a.).**

Statement

Management of patients with haemochromatosis before and during pregnancy is in part determined by liver disease stage and extrahepatic manifestations of the disease **(consensus).**

No dedicated studies have been carried out on maternal and foetal outcomes in pregnant women with haemochromatosis. Considering the natural history of the disease, it is unlikely that the risk of stopping phlebotomy during pregnancy will negatively affect long-term outcome. A normal pregnancy negatively affects the mother's iron balance and corresponds to an iron requirement of ca. 500 mg.[125] Iron deficiency is a risk factor for adverse maternal and foetal outcomes and should therefore be avoided before and during pregnancy.[126] The panel recommends avoiding iron deficiency before and during pregnancy. In patients with haemochromatosis planning to get pregnant, the intensity of therapeutic phlebotomy should be reduced to achieve serum ferritin concentrations of ≥45 μg/L, which is a conservative cut-off for iron deficiency suggested by recent guidelines.[127] As cirrhosis and portal hypertension are risk factors for worse maternal and foetal outcomes, all patients planning to get pregnant should be assessed for the presence of advanced fibrosis or cirrhosis. In advanced fibrosis and cirrhosis, management is primarily determined by disease stage.

What is the first-line treatment in patients with haemochromatosis?

Recommendation

Patients with haemochromatosis and evidence of iron overload should undergo iron depletion therapy **(LoE 2, strong recommendation, strong consensus).**

Statement

The first-line treatment for iron depletion is therapeutic phlebotomy.

Erythrocytapheresis is an alternative to therapeutic phlebotomy and has been shown to be cost effective in the induction phase, because fewer interventions are required, and it can be an option if available. Personalised erythrocytapheresis represents the preferred treatment in selected cases **(consensus).**

The standard of care for the treatment of haemochromatosis is phlebotomy, which reduces body iron accumulation by mobilising iron for erythropoiesis.[130,131] Morbidity and mortality of patients with haemochromatosis is significantly reduced when treatment is initiated before the development of cirrhosis and/or diabetes.[98,132,133]

The available evidence suggests that phlebotomy may improve fatigue, arthralgias and liver function tests, and result in the regression of liver fibrosis and cirrhosis in a subset of patients. Conclusive data regarding the influence of therapeutic phlebotomy on quality of life, liver cancer risk, diabetes or cardiovascular disease is lacking.[133] Therapeutic phlebotomy has been demonstrated to improve survival in clinical studies and cohort studies.[11,117] A recent systematic review of 24 studies, including 5,994 patients, on iron reduction in haemochromatosis highlighted the absence of high-level evidence to support its use.[133]

Treatment comprises two phases, the initial or induction phase, to deplete body iron stores, and a maintenance phase, to prevent iron re-accumulation.[7,134] Phlebotomy is generally safe, effective, and well tolerated. However, given its invasive nature and reliance on attendance at a healthcare facility, concerns exist regarding its inconvenience and potential adverse impact on patients' lives.[135]

Patients with uncomplicated haemochromatosis, *i.e.* without significant organ damage, can be accepted as regular blood donors during the maintenance phase. When possible, blood taken from patients with haemochromatosis should be made available for transfusion.

Patients with haemochromatosis should also continue to undergo routine blood monitoring through their haemochromatosis service or physician. The development of iron deficiency, anaemia and/or an unexplained reduction in the need for phlebotomy during treatment should lead to a complete work up for alternative causes.

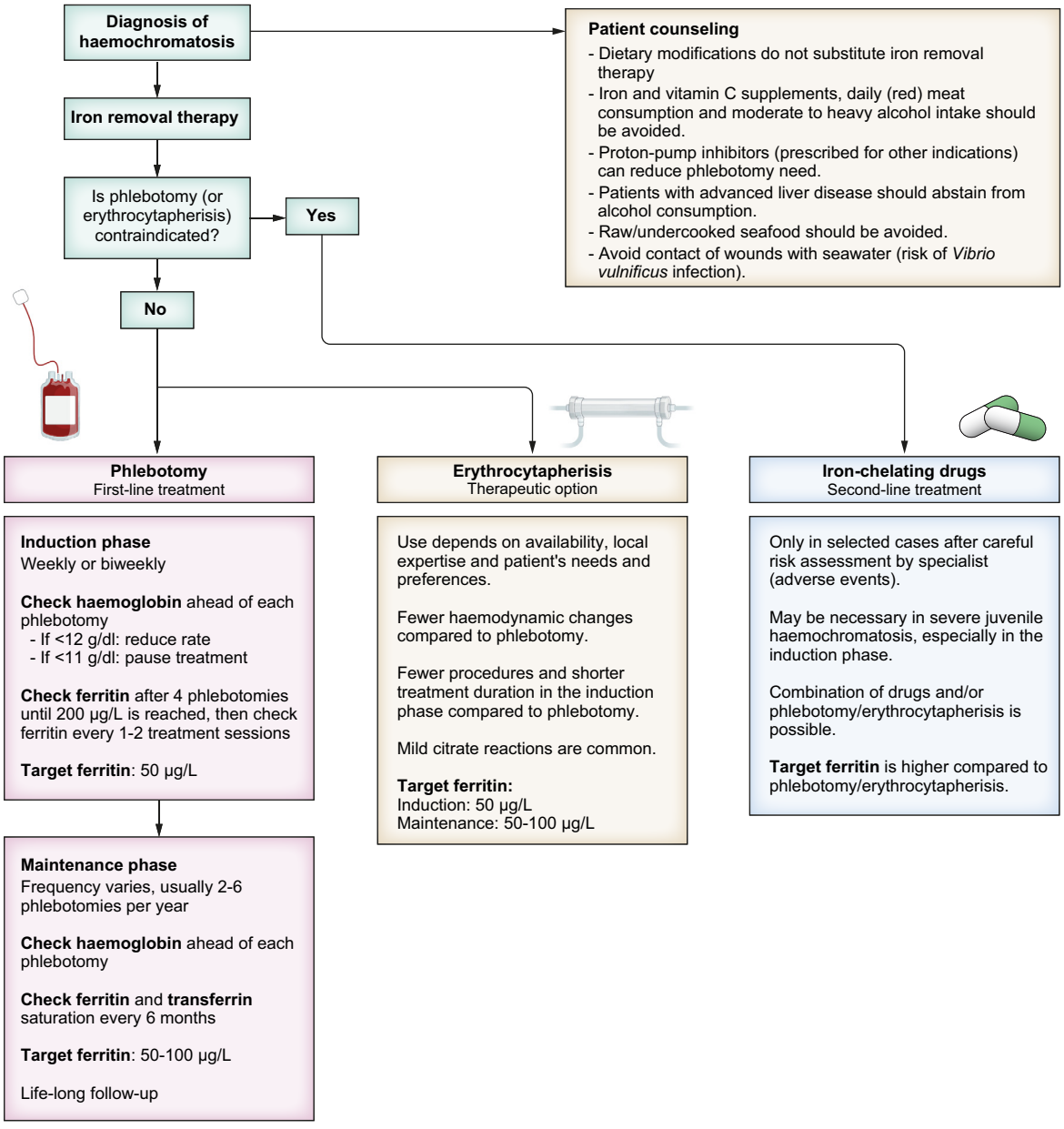

Fig. 5. Treatment of haemochromatosis.

A recent study showed that erythrocytapheresis may reduce fatigue and serum iron parameters more effectively than a sham procedure in patients with haemochromatosis.[12,136] Mild citrate reactions were more common in the treatment group.[124] A small, randomised trial also demonstrated a reduction in procedures required to achieve iron depletion with personalised erythrocytapheresis *vs.* phlebotomy[137] allowing for a reduction in treatment duration and total costs in the induction phase.[125]

An open-label, randomised trial showed that fixed volume erythrocytapheresis instead of phlebotomy results in a faster initial decline in ferritin and a reduced number of procedures, but not in the early achievement of target ferritin or in lower costs. The frequency of discomfort was equally low with the two methods.[136] When a fixed volume of red blood cells is removed, instead of individualised removal based on sex, body weight, total blood volume and haematocrit, erythrocytapheresis becomes more expensive and less competitive.[138] In the maintenance phase, personalised erythrocytapheresis led to a reduction in the number of procedures required but not in the total cost per treatment year.[139]

Since erythrocytapheresis allows for fewer hemodynamic changes compared to phlebotomy and returns valuable blood components, it represents the preferred treatment option in selected cases.[138]

Individuals at risk of developing iron overload (those with at-risk genotypes and increased transferrin saturation, but without increased ferritin levels) may be encouraged to volunteer as regular blood donors, particularly if identified in early adulthood.[140–144]

When should second-line treatment be considered?

Recommendation

If phlebotomy is not possible, iron chelation therapy can be started after careful consideration of risk-benefit ratio **(LoE 4, weak recommendation, consensus).**

Statement

Most evidence in haemochromatosis pertains to oral deferasirox (DFX) as a possible second-line option, which is effective in removing iron, but evidence is weak and DFX should not be used in patients with advanced liver disease. DFX can be associated with gastrointestinal side effects, impairment in kidney function and is not approved for the treatment of haemochromatosis by the European Medicines Agency **(n.a.).**

Phlebotomy is usually very effective, but when it is not feasible or cannot be performed at the desired frequency (and erythrocytapheresis is not feasible or available), alternative ('second-line') treatment options can be necessary. Chelation can be considered in patients with haemochromatosis who have inaccessible veins, needle phobia, concomitant anaemia, life-threatening cardiac iron overload, typically occurring in juvenile haemochromatosis, or conditions where bloodletting can harm.

It should be noted that anaemia is not a characteristic of haemochromatosis. On the contrary, reports on erythroid parameters in haemochromatosis show increased haemoglobin levels, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration[145,146] related to the increased availability of iron for erythropoiesis. Therefore, the eventual finding of anaemia in a patient with haemochromatosis should prompt a thorough investigation of other unrelated causes of anaemia. In patients with advanced disease who poorly tolerate classical phlebotomies (*e.g.* because of low baseline haemoglobin or hemodynamically unstable because of cardiac impairment), personalised (mini)-phlebotomies plus subcutaneous infusion of deferoxamine (DFO) could be considered. Such patients should be referred to specialised centres for evaluation and treatment.

Treatment with DFO (parenteral) and, off-label, with deferasirox (DFX - oral) or deferiprone (DFP - oral) has been described, but these drugs should only be prescribed by those with clinical expertise.

Experience with DFO in haemochromatosis is very limited – it was shown to be a safe alternative to phlebotomy in 3 patients with haemochromatosis.[147] Experience with DFP in haemochromatosis is also very limited. In a retrospective cohort of 71 iron-loaded patients, 32 of whom received DFP, 3/71 had haemochromatosis; all 3 patients with severe cardiac iron overload and haemochromatosis developed haematological toxicity, two in the form of agranulocytosis (one fatal) and one neutropenia.[148]

DFX has been subject to the most study in haemochromatosis: 49 patients with normal renal function received oral DFX (in dispersible tablets) in a phase I/II dose escalation trial, in the absence of transfusion.[149] A number of patients on the 15 mg/kg dose experienced side effects and the authors recommend a starting dose of 10 mg/kg, which reduced median serum ferritin concentration by 75% over a 48-week period and to <250 ng/ml. The tolerability and efficacy of DFX at a dose of 10-15 mg/kg in dispersible tablets was confirmed in a separate phase II study in 10 patients with haemochromatosis.[150]

In juvenile haemochromatosis, phlebotomy has often been used[88] and represents an effective therapeutic tool and the treatment of choice when possible. In the presence of severe clinical manifestations, a combined or alternative chelating therapy is generally required in the unloading phase. In such cases, phlebotomy and chelating agents can be used as alternative, combined, or sequential therapies in the maintenance phase, depending on disease severity, stage, manifestations, and patients' comorbidities and contraindications.[86,151]

In juvenile haemochromatosis, where chelation is typically begun in life-threatening cardiac iron overload, combination chelation therapy with oral DFP and intravenous DFO has been described in several case reports.[152] Reports of HJV-related haemochromatosis with late onset and milder disease were recently described in Asia and Europe,[153–156] suggesting the presence of phenotype modifiers or lower pathogenicity variants, and indicating the efficacy of phlebotomy alone in both the induction and maintenance phases. Treatment options are summarised in Fig. 5.

All iron chelation drugs are contraindicated in pregnancy and dose adjustment is required in renal failure. Specific drug contraindications and possible side effects must be considered and discussed with patients. Careful monitoring is required, tailored to the iron chelator used – for DFX, liver and renal function should be monitored monthly, with an audiological and ophthalmological review performed prior to starting treatment and annually thereafter.

By reducing non-heme iron absorption through increased gastric pH, proton pump inhibitors (PPIs) could be a useful addition in some patients. Indeed, one study reported that the use of PPIs significantly reduced the need for bloodletting during the maintenance phase in patients with HFE-related haemochromatosis.[157] Since they are not devoid of side effects and have not been studied in the induction phase, PPIs should be considered as a supportive treatment in specific cases and not as a first- or second-line therapeutic tool. In patients receiving PPIs for other reasons, a reduction in the need for phlebotomy can be expected.

What are the treatment targets?

Recommendations

Phlebotomy encompasses an induction and maintenance phase. During the induction phase, phlebotomy should be performed weekly (or fortnightly) until iron stores are depleted. The target for iron depletion during induction is a serum ferritin of 50 μg/L, but not lower to avoid iron deficiency **(LoE 4, strong recommendation, strong consensus).**

In the maintenance phase, serum ferritin can be maintained with some flexibility in the range of 50-100 μg/L **(LoE 4, weak recommendation, n.a.)**

Guidelines and recommendations from scientific societies and expert groups indicate similar but not equal therapeutic targets. The guideline prepared by EASL in 2010 recommended a serum ferritin <50 μg/L in the induction phase and 50-100 μg/L in the maintenance phase for patients with HFE-haemochromatosis, while in 2011 the American Association for the Study of Liver Diseases promoted a target level of serum ferritin of between 50 and 100 μg/L in both phases.[158] National guidelines have also been published. The Dutch guideline from 2007 recommended a target serum ferritin <50 μg/L in the induction phase and within reference value in the maintenance phase,[159] while, in 2016, the Haemochromatosis Expert Panel defined a target serum ferritin level of 50–100 μg/L for all 'on-treatment' patients.[160] In 2018, the British Society for Haematology recommended serum ferritin 20–30 μg/L and transferrin saturation <50% in the induction phase and serum ferritin <50 μg/L and transferrin saturation <50% in the maintenance phase in all fit patients.[95] The Danish 2019 guideline promoted serum ferritin <100 μg/L in the induction phase, and serum ferritin 50-100 μg/L and transferrin saturation <60% in the maintenance phase.[161] The American College of Gastroenterology recently defined a 50–100 μg/L single target serum ferritin level for patients with haemochromatosis.[162,163]

A recent practical set of recommendations on therapeutic aspects of HFE-related haemochromatosis was published in 2018 by an international group of experts, based on the published studies and guidelines, and proposes a target ferritin of 50 μg/L for the induction phase, and a range of 50-100 μg/L for the maintenance phase.[164] Experts of the Delphi panel commented that during the maintenance phase, treatment targets should be more relaxed and ferritin in the ranges of <200 μg/L for women and <300 μg/L for men could be tolerated. Aiming ferritin concentrations down to 50 μg/L during the maintenance phase is poorly tolerated by elderly patients. These more relaxed treatment targets more likely reflect clinical practice but are mainly based on expert opinion and have not been investigated in clinical studies.

The volume and frequency of phlebotomies required to reach these goals are usually 400–500 ml weekly or every 2 weeks during the induction phase, depending on body weight and patient tolerance, and every 1–4 months during the maintenance phase, depending on the patient's iron status.[7,134,164] The iron accumulation rate in haemochromatosis after depletion varies widely among patients, with estimates that, on average, serum ferritin rises by around 100 μg/L in a year without treatment.[165]

Serum haemoglobin should always be monitored during both induction and maintenance phases, at the time of each bloodletting session. If haemoglobin concentrations are <12 g/dl, the frequency of phlebotomy should be decreased and in specific cases a reduction in volume should be considered. When haemoglobin is <11 g/dl, phlebotomy should be discontinued and blood tests and clinical evaluation should be reassessed at a later time.

Serum ferritin should always be monitored to ensure that the target value is achieved and maintained and to avoid overtreatment (possibly leading to unnecessary bloodletting or iron deficiency). Depletion of iron stores (serum ferritin <50 μg/L) led to a further reduction in hepcidin levels in patients with HFE-haemochromatosis even at a time remote from phlebotomy,[166] indicating that the modulation of hepcidin by body iron depletion is preserved in such patients. Concordantly, phlebotomy resulting in serum ferritin <20 μg/L significantly increased dietary non-heme iron absorption in a small group of patients with idiopathic haemochromatosis.[167] Moreover, symptomatic iron deficiency can develop in patients with haemochromatosis.[168] During the induction phase, serum ferritin should be measured every month (or after every 4th phlebotomy). When levels decrease below 200 μg/L, ferritin should be quantified every 1-2 sessions down to the target level. During the maintenance phase, serum ferritin should be monitored every 6 months to adapt the treatment schedule and ensure that ferritin remains within the target range. Unexpected changes in serum ferritin or transferrin saturation levels should always be investigated, as significant fluctuations are not a feature of haemochromatosis.

An open issue is the monitoring of transferrin saturation, since evidence-based target levels are lacking. Furthermore, transferrin saturation may remain increased even when serum ferritin levels are within the target range in patients with haemochromatosis. Observational data suggest that general and joint symptoms may be related to long-term exposure to

transferrin >50% regardless of serum ferritin being <50 μg/L in HFE-related haemochromatosis.[169]

It is advisable to periodically check plasma folate and plasma cobalamin, especially in patients who require numerous venesections; if necessary, vitamin supplements should be administered.

What are the dietary recommendations in haemochromatosis?

Recommendations

Dietary modifications should not substitute for iron removal therapy **(LoE 5, strong recommendation, strong consensus).**

Iron supplementation should be avoided. Iron fortified food should be avoided where possible **(LoE 5, strong recommendation, consensus).**

Supplemental vitamin C should be avoided, especially before iron depletion **(LoE 4, weak recommendation, consensus).**

Red meat consumption should be limited **(LoE 4, weak recommendation, n.a.).**

Alcohol intake should be restricted, during the iron depletion phase of treatment. Patients with iron overload and/or liver abnormalities should avoid or consume very little alcohol. Patients with cirrhosis should abstain from alcohol consumption **(LoE 4, weak recommendation, consensus).**

Statements

Fruit juices and fruit, especially citrus fruits, are best consumed in moderation, and not in combination with other foods **(consensus).**

Alcohol is a carcinogen and has been associated with increased risk of several malignancies, including liver cancer **(n.a.).**

In patients with haemochromatosis and iron overload, direct handling and consumption of raw or undercooked shellfish and wound exposition to seawater has been associated with a rare but serious systemic bacterial infection by *Vibrio vulfinicus*, and other siderophilic pathogens in certain geographical regions **(strong consensus).**

Phlebotomy is the mainstay of therapy. Dietary and lifestyle modifications can help to reduce iron accumulation and to prevent or limit organ damage and disease complications.

Patients frequently ask about dietary restrictions. Moreover, if not advised, they can sometimes independently implement unhealthy dietary adjustments with the goal of reducing iron intake. Therefore, diet and lifestyle recommendations should be discussed when a diagnosis of haemochromatosis is made.

In general, patients should have a healthy diet as for individuals without haemochromatosis and avoid iron and vitamin C supplementation and heavy alcohol consumption.

Vitamin C (ascorbic acid) is a powerful enhancer of non-heme iron absorption and has physiological antioxidant, chelating, and coenzyme activities.[170] In contrast to its antioxidant activity and use, ascorbic acid under certain conditions can also act as a pro-oxidant and a source of free radicals. Interactions between iron and ascorbic acid are complex and the resulting implications for health and disease states are not yet fully elucidated.[170] In iron-overloaded patients with thalassemia major, oral supplementation of vitamin C has been associated with acute deterioration of cardiac function, likely due to iron mobilisation from reticuloendothelial stores, increased iron availability, and free radical generation.[171] Sporadic case reports suggested that high-dose oral vitamin C can accelerate iron deposition and favour deterioration of heart disease in severe haemochromatosis.[172,173] Although data on the effects of supplemental vitamin C in patients with haemochromatosis are sparse, it is prudent to counsel patients to avoid supplemental vitamin C (especially when they are iron overloaded and during the induction phase) and to discuss any eventual supplementation with a physician, limiting the dosage to 500 mg daily.

Heme iron is well absorbed and its bioavailability is little affected by meal composition. Individuals with haemochromatosis show a greater dietary iron absorption, for both heme and non-heme iron, and a weaker regulatory feedback mechanism on iron absorption at high serum ferritin concentrations, particularly for heme iron, in comparison to healthy controls.[174] Moreover, meat favours non-heme iron absorption when added to a vegetable meal. In order to reduce iron intake, meat (especially read meat) consumption should be limited. Fruit and vegetable intake does not need to be restricted. However, due to their vitamin C content, fruit juices and fruit (mainly citrus fruits) are best consumed alone (*i.e.* not in combination with other foods). Conversely, certain diet components (*e.g.* black tea) may have an inhibitory effect on iron absorption in patients with haemochromatosis.[174]

Overall, dietary modifications may provide an additional measure to reduce long-term iron accumulation and the number of required phlebotomies, but data on the clinical and quality-of-life benefit in patients with haemochromatosis are limited.[174]

Chronic high alcohol consumption influences serum iron indices and liver iron content and can accelerate fibrosis and increase the risk of cirrhosis, HCC and liver-related mortality in a variety of chronic liver diseases.[175–178] The effects of low to moderate alcohol consumption are less clear.[176,177] In addition, clinical studies investigating the contribution of alcohol to the progression or severity of chronic liver diseases have limitations.[177]

Chronic excess alcohol intake has been associated with increased risk of fibrosis, cirrhosis and liver cancer in patients with haemochromatosis.[176–178] Both alcohol-related liver disease and hepatic iron overload are individually associated with significant oxidative stress, lipid peroxidation, fibrogenic processes, and carcinogenesis.

In a meta-analysis from 2010, an increased risk of mortality from cirrhosis was reported among men and women drinking 12–24 g of ethanol/day. Among women, a significant increase was also seen for those drinking up to 12 g/day. Moreover, alcohol is a recognised carcinogen. Indeed, alcohol

consumption has been associated with an increased risk of several malignancies, including liver cancer, and in some of them the risk begins at doses as low as 10 g/day.[179] In rodent models, alcohol downregulates hepcidin transcription in the liver via oxidative stress, irrespective of iron overload, thus abrogating the protective effect of hepcidin against iron accumulation.[175]

Alcohol history and current consumption should always be assessed in patients with suspected or diagnosed iron overload disorders. Considering the fibrogenic and carcinogenic effect of alcohol and iron, patients with haemochromatosis should be generally advised to avoid or reduce alcohol consumption. If alcohol is consumed, daily intake should be below the threshold for alcohol-related liver disease[178] and further restrictions should be tailored to disease severity, organ involvement, liver damage, and age, sex and comorbidities of the patients. Alcohol consumption should be avoided or limited to occasional and very small amounts in the presence of iron overload, organ disease, liver abnormalities, and other chronic liver diseases. Heavy alcohol intake should always be discouraged.

Vibrio vulnificus is a globally distributed, gram-negative pathogen that grows as normal marine flora (mainly in warm coastal waters) and can contaminate seafood. *Vibrio vulnificus* causes severe to life-threatening infections (skin and soft-tissue infections and primary septicaemia) in susceptible patients, including those with haemochromatosis and other iron overload conditions, with a mortality rate of >50% in those with fulminant sepsis.[180,181] Clinical reports can be explained by *in vitro* and *in vivo* studies showing that high iron levels trigger very rapid bacterial growth, that plasma iron concentration influences bacterial replication and dissemination, and that the hepcidin-mediated acute hypoferremia in response to *Vibrio vulnificus* is an important host defence mechanism against this siderophilic pathogen.[182] Thus, hepcidin deficiency and the high levels of circulatory iron typical of haemochromatosis expose patients (especially if iron overloaded) to the risk of severe *Vibrio vulnificus* infections. In patients with normal serum ferritin and normal transferrin saturation the risk of *Vibrio vulnificus* infection and a severe disease course is probably mitigated.

Appendix: Delphi round agreement on the statements and recommendations of the present CPGs

Recommendation/statement	Consensus
Individuals with clinical and biochemical signs of haemochromatosis, elevated transferrin saturation and high serum ferritin concentrations, or otherwise unexplained persistently elevated transferrin saturation should be genetically tested for haemochromatosis after informed consent for genetic testing has been obtained.	100%
Patients with increased liver iron evident on liver biopsy or MRI should be clinically assessed and biochemically tested for haemochromatosis (serum ferritin and transferrin saturation).	100%
Adult individuals with a positive family history of first-degree relatives with haemochromatosis should be genetically tested for haemochromatosis after informed consent for genetic testing has been obtained.	100%
The first step in testing for haemochromatosis is the assessment of serum iron parameters, which should include transferrin saturation and serum ferritin.	100%
The concentration of serum iron and transferrin or total iron binding capacity can provide additional information for the differential diagnosis.	87%
Measuring hepcidin is not recommended.	100%
Genotyping for p.C282Y in HFE should be carried out in Individuals of European origin with biochemical evidence of iron overload (females with transferrin saturation >45% and serum ferritin >200 μg/L and males with transferrin saturation >50% and ferritin >300 μg/L, or otherwise unexplained persistently elevated transferrin saturation) with or without clinical signs or symptoms suggestive of haemochromatosis.	100%
Adult (>18 years of age) first-degree relatives of patients with p.C282Y homozygous haemochromatosis should be tested for the p.C282Y variant in HFE.	100%
Genotyping for p.H63D can be performed in special clinical situations. In combination with p.C282Y and usually other acquired risk factors, p.H63D is associated with a higher risk of mild iron overload.	n.a.
• Genotyping for p.H63D is not generally suggested to guide treatment, but the value of genotyping for this variant remains controversial.	n.a.
The management of patients with p.C282Y/p.H63D compound heterozygosity or p.H63D homozygosity should be guided by their phenotypic presentation and the presence of additional risk factors, not the genotype alone.	100%
Patients who are compound heterozygous for p.C282Y/p.H63D or homozygous for p.H63D with confirmed iron overload should be investigated for other causes of iron overload.	93%
Patients who are compound heterozygous for p.C282Y/p.H63D or homozygous for p.H63D with confirmed iron overload (by MRI or liver biopsy) may be treated with phlebotomy, but this treatment decision requires individualised clinical assessment.	100%
In patients with an unclear cause of hyperferritinemia, biochemical iron overload (increased transferrin saturation and ferritin) or positive liver iron staining, MRI should be used to quantify hepatic iron concentrations and to assess extrahepatic organ involvement.	100%
Cardiac MRI can be performed in patients with haemochromatosis and signs of heart disease, and in juvenile forms of haemochromatosis.	100%
• MRI is helpful for detection, non-invasive quantification of iron, and to study the distribution of iron in the liver, spleen, pancreas, heart, and brain in patients with suspicion or diagnosis of iron overload disorder. In patients with suspected aceruloplasminemia, MRI of the brain can also provide important additional information.	100%

(*continued on next page*)

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Recommendation/statement	Consensus
Liver biopsy aims to assess the presence of cirrhosis; therefore, in patients with an otherwise clear diagnosis of cirrhosis, liver biopsy is not recommended.	87%
Liver biopsy can be performed to assess liver fibrosis if serum ferritin is higher than 1,000 μg/L or if liver enzymes are increased.	100%
Liver biopsy is not recommended for the diagnosis of hepatic iron overload.	88%
All patients with haemochromatosis should be non-invasively assessed for the presence of liver fibrosis at diagnosis to guide appropriate treatment and follow-up.	100%
Transient elastography can be used to rule out advanced fibrosis in patients with haemochromatosis if liver stiffness is ≤6.4 kPa.	100%
• FIB-4 is the serum-based non-invasive marker which is best evaluated for the assessment of fibrosis stage in patients with chronic liver diseases, but the available evidence is limited in patients with haemochromatosis.	100%
• In patients with ferritin <1,000 μg/L, normal transaminases and no liver enlargement, the risk of advanced liver fibrosis is very low.	77%
Clinicians should clinically evaluate patients with haemochromatosis for extrahepatic manifestations including skeletal (joint pain, arthritis, osteoporosis, fractures) and endocrine (diabetes) manifestations, and reproductive or sexual dysfunction (erectile dysfunction, loss of libido or amenorrhea).	100%
Patients with severe iron overload should be evaluated for arrhythmia and cardiac dysfunction (electrocardiogram [ECG] and echocardiography).	100%
Patients with severe haemochromatosis and signs or symptoms of heart disease (conduction disease and/or contractile dysfunction) should be investigated with cardiac MRI for iron quantification without delaying treatment.	100%
Patients with juvenile haemochromatosis should be investigated for cardiac involvement, including myocardial iron quantification by MRI.	100%
Patients with haemochromatosis and cirrhosis (on biopsy METAVIR F4; Ishak scoring system stage 6 or on elastography) should undergo HCC screening every 6 months, regardless of iron depletion.	100%
HCC screening every 6 months can be suggested in patients with haemochromatosis and advanced fibrosis (*i.e.* bridging fibrosis; METAVIR F3; Ishak scoring system stages 4-5), regardless of iron depletion.	86%
Patients with haemochromatosis and regression of advanced fibrosis or cirrhosis to a stage of F2 or less after treatment should continue HCC screening, but the surveillance interval may be individualised.	100%
Surveillance of HCC should be performed by experienced personnel using abdominal ultrasound every 6 months.	100%
When ultrasound evaluation is technically suboptimal, HCC surveillance should be performed by MRI or CT.	93%
Imaging-based surveillance of HCC can be facultatively performed in combination with serum alpha-fetoprotein (AFP) every 6 months.	85%
• HCC risk in patients with haemochromatosis and pretreatment advanced fibrosis is modified by the presence of additional risk factors for liver cancer such as alcohol consumption, type 2 diabetes mellitus, and viral hepatitis, but validated risk scores for HCC are not available to tailor surveillance in patients with haemochromatosis.	93%
Young individuals with biochemical evidence and clinical manifestations of haemochromatosis (liver disease, amenorrhea, hypogonadism, cardiomyopathy) should be tested for rare haemochromatosis gene variants.	100%
Patients with evidence of significant, unexplained iron overload should be referred for assessment by a specialist in iron disorders.	100%
Adult first-degree relatives of patients with rare variants in haemochromatosis genes should be tested for these variants; particular focus should be given to siblings as they are at the highest risk of haemochromatosis.	100%
In patients with haemochromatosis planning to get pregnant, iron deficiency should be avoided.	100%
In pregnant women with mild to moderate iron overload without signs of advanced liver disease, decisions regarding therapeutic phlebotomy can be individualised but phlebotomy can be paused for the duration of pregnancy in most patients.	n.a.
• Management of patients with haemochromatosis before and during pregnancy is in part determined by liver disease stage and extrahepatic manifestations of the disease.	93%
Patients with haemochromatosis and evidence of iron overload should undergo iron depletion therapy.	100%
• The first-line treatment for iron depletion is therapeutic phlebotomy. Erythrocytapheresis is an alternative to therapeutic phlebotomy and has been shown to be cost effective in the induction phase, because fewer interventions are required, and it can be an option if available. Personalised erythrocytapheresis represents the preferred treatment in selected cases.	94%
If phlebotomy is not possible, iron chelation therapy can be started after careful consideration of risk-benefit ratio.	94%
• Most evidence in haemochromatosis pertains to oral deferasirox (DFX) as a possible second-line option, which is effective in removing iron, but evidence is weak and DFX should not be used in patients with advanced liver disease. DFX can be associated with gastrointestinal side effects, impairment in kidney function and is not approved for the treatment of haemochromatosis by the European Medicines Agency.	n.a.
Therapeutic phlebotomy encompasses an induction and maintenance phase. During the induction phase phlebotomy should be performed weekly (or fortnightly) until iron stores are depleted. The target for iron depletion during induction is a serum ferritin of 50 μg/L, but not lower to avoid iron deficiency.	100%
In the maintenance phase, serum ferritin can be maintained with some flexibility in the range of 50-100 μg/L.	n.a.
Dietary modifications should not substitute for iron removal therapy.	100%
Iron supplementation should be avoided. Iron fortified food should be avoided where possible.	94%
Supplemental vitamin C should be avoided, especially before iron depletion.	88%
Red meat consumption should be limited.	n.a.
Alcohol intake should be restricted, during the iron depletion phase of treatment. Patients with iron overload and/or liver abnormalities should avoid or consume very little alcohol. Patients with cirrhosis should abstain from alcohol consumption.	94%
• Fruit juices and fruit, especially citrus fruits, are best consumed in moderation, and not in combination with other foods.	85%
• Alcohol is a carcinogen and has been associated with increased risk of several malignancies, including liver cancer.	n.a.
• In patients with haemochromatosis and iron overload, direct handling and consumption of raw or undercooked shellfish and wound exposition to seawater has been associated with a rare but serious systemic bacterial infection by *Vibrio vulfinicus,* and other siderophilic pathogens in certain geographical regions.	93%

Abbreviations

AFP, alpha-fetoprotein; CPG, clinical practice guideline; DFO, deferoxamine; DFP, deferiprone; DFX, deferasirox; EASL, European Association for the Study of the Liver; HAMP, hepcidin; HCC, hepatocellular carcinoma; HJV, hemojuvelin; HR, hazard ratio; PPIs, proton pump inhibitors; TFR2, transferrin receptor 2.

Conflict of interest

Please refer to the accompanying EASL disclosure forms for details.

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Supplementary data

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