A Review of New Concepts in Iron Overload

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Abstract: Iron overload disorders are conditions that can lead to increased body iron stores and end-organ damage in affected organs. Increased iron deposition most commonly occurs in the liver, heart, endocrine system, joints, and pancreas. Iron overload disorders may be caused by genetic or acquired causes (transfusion, dyserythropoiesis, and chronic liver disease). The *HFE* gene C282Y homozygous mutation is the most common cause of hereditary hemochromatosis (HH). Other genes implicated in HH include *TFR2*, *HAMP, HJV,* and *SLC40A1*. In the past 2 decades, there have been major advances in the understanding of genetic iron overload disorders. Furthermore, new novel techniques to measure iron content in organs noninvasively, as well as new therapeutic options for the treatment of HH, are currently under development. This article focuses on the latest concepts in understanding, diagnosing, and managing genetic iron overload disorders, particularly HH.

Keywords
Iron overload, hemochromatosis, hepcidin mimetics, *HFE*

Iron is an essential micronutrient for fundamental cell and organ function.[1] Iron has unique electrochemical properties and can undergo redox switching between ferric and ferrous forms.[2] This property makes it an ideal factor for many biologic processes, but this also sets the basis for its toxicity if not bound to iron-storage proteins.[2] Unbound iron contributes to the generation of free radicals and results in oxidative damage.[2] Iron overload disorders can occur owing to genetic disorders or acquired conditions such as transfusion, dyserythropoiesis, and chronic liver disease.[3] The most common genetic iron overload disorder is caused by the homozygous C282Y mutation in the *HFE* gene and is referred to as hereditary hemochromatosis (HH) type 1.[4] Other genes involved in iron metabolism, such as *TFR2, HAMP, HJV,* and *SLC40A1,* have also been implicated as causes of HH.[5,6] This article reviews genetic iron overload disorders with particular emphasis on new updates that have improved the understanding, diagnosis, and management of patients with HH.

Iron Metabolism

Iron metabolism is tightly regulated to maintain adequate iron stores.[7] Adequate iron is needed for vital cellular processes; however, excessive iron can lead to oxidative damage.[1,2] Iron metabolism is therefore

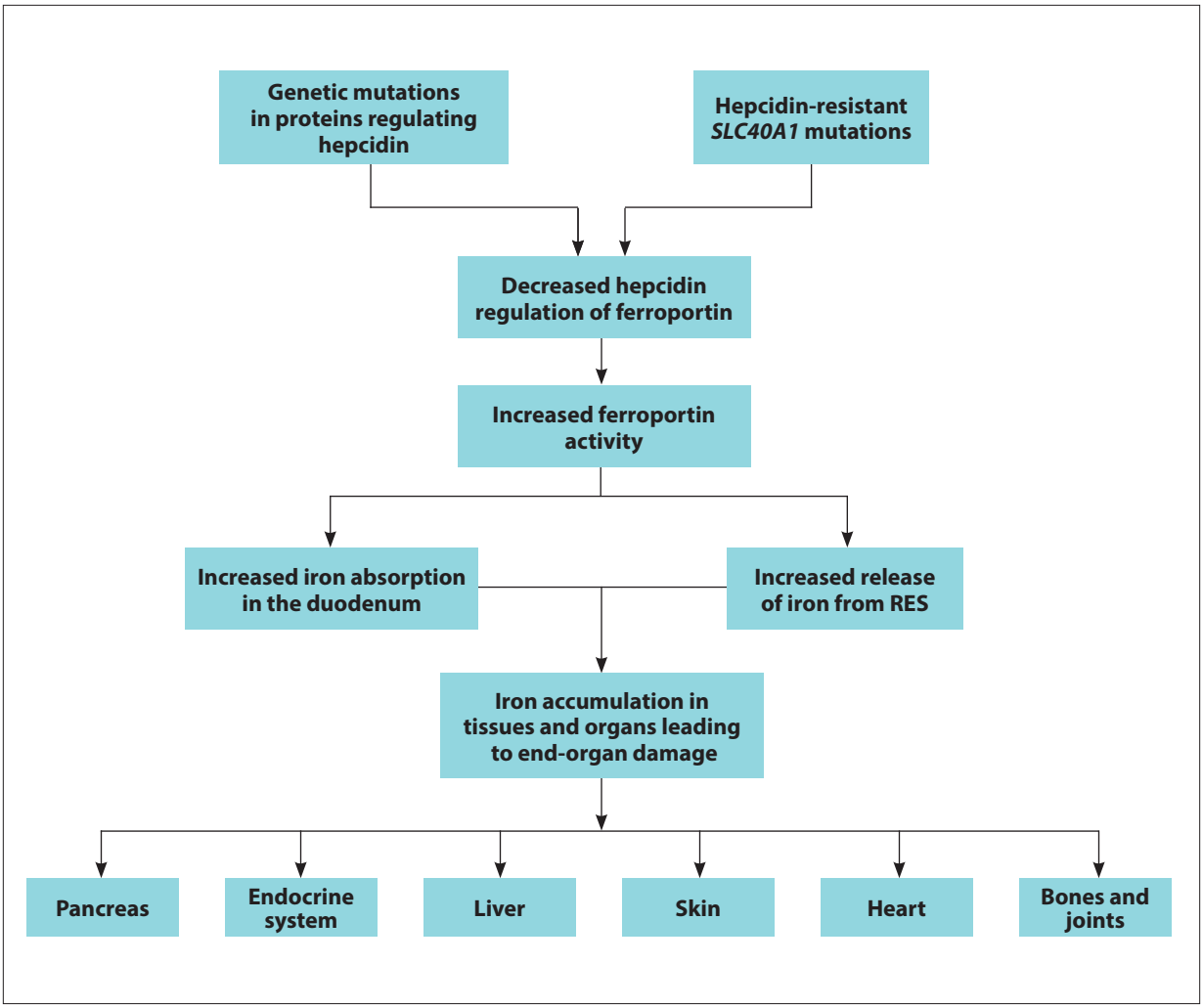

Figure 1. Pathophysiology of iron overload in patients with hemochromatosis.
RES, reticuloendothelial system.

orchestrated by maintaining a balance between iron absorption, storage, and iron release from intracellular components in the hepatocytes and reticuloendothelial system (RES).[7] Under normal conditions, dietary ferric iron is reduced to a ferrous form by duodenal cytochrome B, present in the brush border protein of enterocytes. It then enters the apical brush border using divalent metal transporter 1.[8,9] A second transporter, ferroportin (FPN), transports iron from the basolateral membrane to circulation in conjunction with hephaestin, a ferroxidase, and is subsequently transported into circulation bound to transferrin.[7] Transferrin-bound iron is taken up by various cells using endocytosis via the transferrin receptor.[10]

FPN is the only transmembrane protein responsible for the export of iron and is present in macrophages, hepatocytes, and enterocytes.[11] Hepcidin, a key regulator of iron homeostasis, is produced in the liver and promotes internalization and degradation of FPN, leading to a reduction in iron absorption and release from macrophages.[12,13] The bone morphogenetic protein (BMP)-mothers against decapentaplegic homolog (SMAD) pathway plays an essential role in modulating iron homeostasis via regulation of hepcidin expression.[14] BMP2 and BMP6 are produced by sinusoidal endothelial cells and form an integral component of hepcidin signaling in response to increased iron in the tissues.[15]

BMP6 has also been shown to bind to hemojuvelin, a part of the iron-sensing complex that includes *HFE, HJV,* and *TFR2*.[15-17] Mutations of proteins affecting the regulation of *HFE* decrease the ability of hepatocytes to sense circulating iron and result in inappropriate hepcidin secretion relative to iron stores, and lead to iron overload.[18] Before discussing individual causes of genetic iron overload disorders, it is pertinent to understand that there

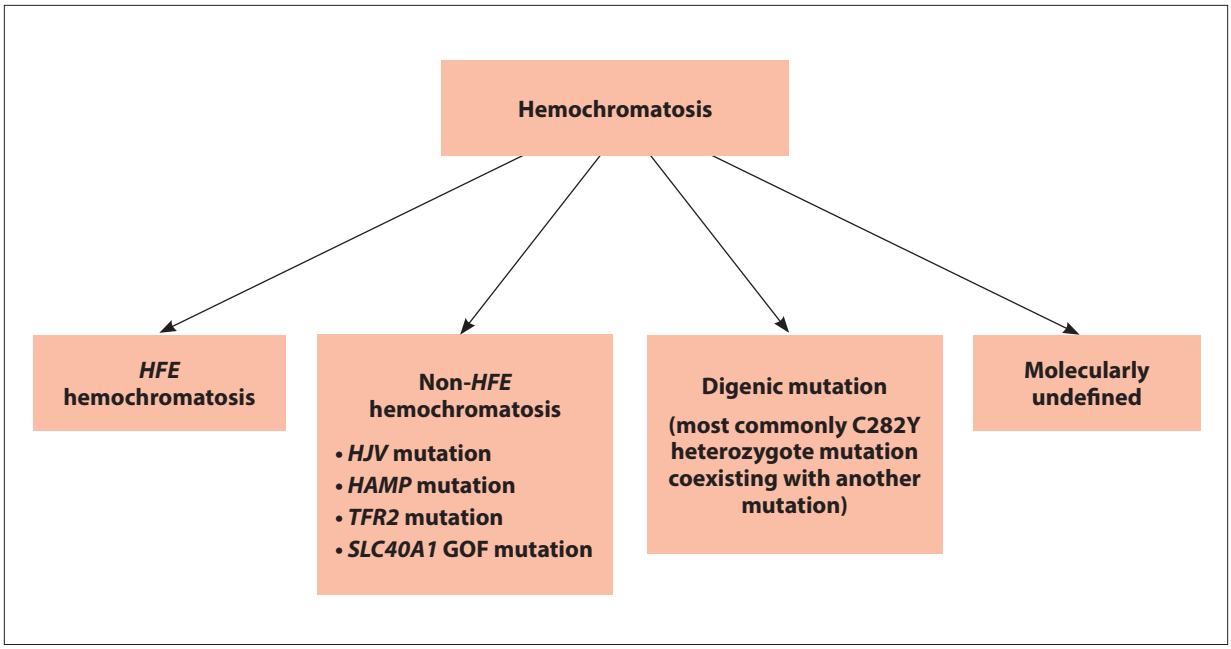

Figure 2. New classification of hemochromatosis according to the BIOIRON Society.
GOF, gain of function.

is no active mechanism for the excretion of excess iron and only a small amount (1-2 mg) of iron is lost from the sloughing of gastrointestinal cells or menses.[19] As a result, removing excess iron via phlebotomy continues to be the best therapeutic option for patients with iron overload disorders.

Genetic Iron Overload Disorders

Mutations in the hepcidin-FPN axis cause HH.[20] The most common mutation is the C282Y mutation in the *HFE* gene.[21] Other causes of HH include mutations in *TFR2, HJV,* or *HAMP,* and a gain-of-function mutation of *SLC40A1*.[21] These mutations also lead to iron overload by decreasing the production/activity of hepcidin or the sensitivity of hepcidin to FPN. HH caused by mutations in *HJV* and *HAMP* has been associated with the lowest hepcidin levels and manifests with the most marked iron overload; it is also referred to as juvenile hemochromatosis (type 2 HH) because of clinical presentation with severe iron overload early in life. The pathophysiology of iron overload in HH is described in Figure 1. Recently, the BIOIRON Society described patients with iron overload who tested positive for 2 different mutations in 2 different genes as having *digenic mutation* and classified it as distinct entities.[20] The Society also described cases that generally do not display variants in any of the classical HH genes as *molecularly undefined*.[20] This information is presented in Figure 2.

HFE Hemochromatosis

The *HFE* gene contributes to the regulation of hepcidin expression.[21] The C282Y mutation in the *HFE* gene leads to inappropriately low levels of hepcidin. This results in uncontrolled circulatory iron pool expansion and progressive tissue iron accumulation, among those patients expressing the phenotype.[22] This gene mutation is highly prevalent in Whites. The prevalence of C282Y homozygosity has been reported to be as high as 1:200 to 1:300.[23] The clinical penetrance of C282Y homozygotes is low, and it has been reported that half of the patients with this mutation will have no signs of iron overload.[23] Clinical expression is higher in male homozygotes than premenopausal women, likely owing to reduced body iron stores resulting from menstruation, pregnancy, and hormonal factors.[24]

In asymptomatic individuals, an isolated high serum iron level or elevated transferrin saturation (TSAT), with or without increased serum ferritin levels, may be the initial presentation.[25] Some patients may present with nonspecific symptoms such as fatigue, malaise, and arthralgia.[26] Patients with delayed diagnosis can present with liver disease, diabetes, endocrine failure, joint inflammation, heart disease, and skin pigmentation changes.[26]

An elevated TSAT has been noted to have a high sensitivity for the identification of HH and is elevated before the development of iron overload.[26] In symptomatic patients with suspected iron overload and elevated ferritin levels, normal TSAT makes the diagnosis

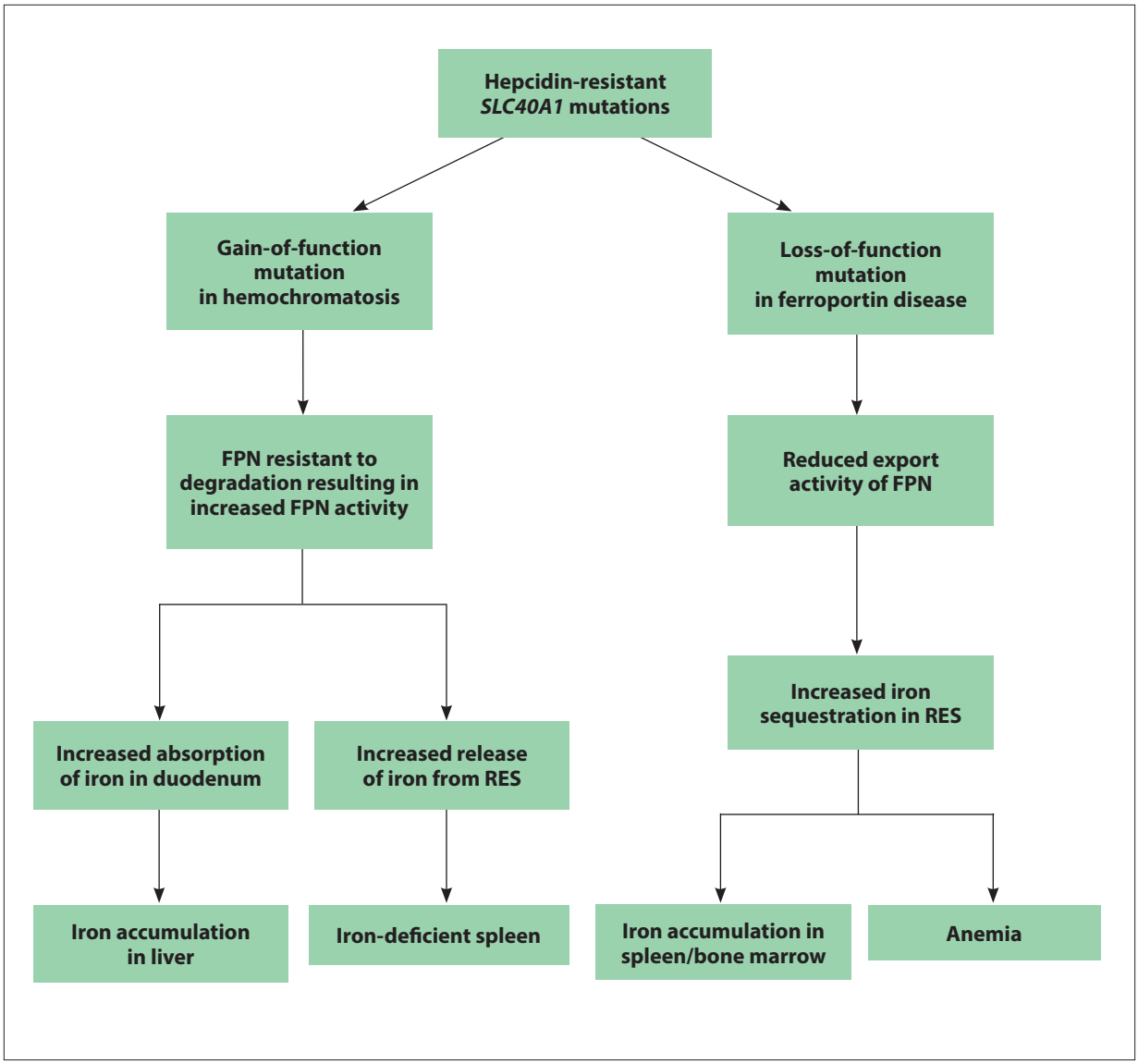

Figure 3. Mechanism of iron overload in hereditary hemochromatosis (type 4) and ferroportin disease.
FPN, ferroportin; RES, reticuloendothelial system.

of *HFE* hemochromatosis less likely, and an extensive workup should be pursued to evaluate for other causes of hyperferritinemia.[26]

Non-HFE *Hemochromatosis*

This group includes HH associated with mutations in the *HJV* (hemojuvelin), *TFR2* (transferrin receptor 2), or *HAMP* (hepcidin) genes, as well as the gain-of-function mutation of *SLC40A1* (FPN).[21] These mutations reduce hepcidin synthesis or sensitivity, resulting in iron overload.[21] Juvenile hemochromatosis associated with *HJV* mutations results in iron accumulation at an accelerated rate leading to the early age of diagnosis, with cardiomyopathy and endocrine failure often present at diagnosis.[27,28] Gain-of-function mutation of *SLC40A1* results in phenotypic expression similar to HH, whereas loss-of-function mutation of the *SLC40A1* gene results in ferroportin disease (FD).[29] FD is described in detail in the following section. The mechanism of iron deposition in HH caused by a gain-of-function mutation in *SLC40A1* (type 4 HH) and FD is presented in Figure 3.

Other Iron Overload Disorders

Genetic iron overload disorders that are not caused by decreased hepcidin levels or resistance of hepcidin, but caused by other mutations, include FD and hereditary

aceruloplasminemia. TSAT is a key differentiating factor between these disorders and HH. Patients with HH have high TSAT, whereas patients with non-HH iron overload disorders have normal TSAT.

FD follows an autosomal dominant pattern of inheritance and is one of the most frequent causes of non-HH genetic iron overload disorders.[29] The loss-of-function mutation in the *SLC40A1* impairs the export function of FPN.[29] This prevents the release of iron from the RES and leads to a greater increase in the splenic iron content and a moderate increase in the hepatic iron content.[29,30] Because this disease is not associated with greatly increased body iron stores but rather sequestration in the RES compartment, it has been suggested to have milder clinical manifestations than HH.[29]

Aceruloplasminemia is a rare autosomal recessive disease with an absence or dysfunction of ceruloplasmin due to a mutation.[31] Ceruloplasmin is a ferroxidase involved in the oxidation of ferrous to ferric iron, the form in which it is transported by transferrin.[31] Ceruloplasmin deficiency prevents iron transport and leads to excess iron accumulation in the pancreas, liver, and nervous tissue.[32] This is the only condition among genetic iron overload disorders that has been clearly shown to affect nerve tissue.[32] Patients present with microcytic hypochromic anemia, elevated ferritin, decreased copper, and no to minimal ceruloplasmin in serum.[30] Genetic testing should be performed only if aceruloplasminemia has been confirmed.[30] Further studies are needed to elucidate the pathophysiologic mechanisms for iron overload in this disorder because, based on the proposed mechanism, this condition should lead to an FD-like iron distribution.[30]

Diagnosis

Previously, liver biopsy was the gold standard for diagnosing HH.[33] With improvement in the understanding of genetic iron overload disorders, the diagnosis is increasingly based on genetic testing, the use of novel imaging techniques for the assessment of liver iron content, and estimation of the hepatic fibrosis stage.

Laboratory Abnormalities

TSAT is the earliest biochemical abnormality in patients with HH.[26] A TSAT greater than 45% has been reported to identify the majority of C282Y homozygotes in patients who express the phenotype.[34] A normal TSAT rules out HH in most cases.[26] Another common abnormality in patients with HH is elevated serum ferritin, although studies have reported that elevated serum ferritin levels lack specificity for the diagnosis of HH.[35] However, elevated ferritin (>1000 ng/mL) has been reported to be a predictor of advanced fibrosis in patients with HH.[35,36] In patients with elevated ferritin but normal TSAT and negative *HFE* genetic testing, workup should include evaluation for chronic liver conditions, hematologic disorders, and other genetic iron overload disorders.[3]

Genetic Testing and Screening

In 1996, Feder and colleagues identified a homozygous mutation in the *HFE* gene in 83% of patients with HH.[37] Currently, genotyping for *HFE* mutations is a part of the standard evaluation for patients suspected to have HH.[26,35] Three common mutations have been described in the *HFE* gene (C282Y, H63D, and S65C).[38,39] Among these, the C282Y homozygous mutation is the only one clearly associated with the clinical expression of HH.[38] Neither homozygous nor heterozygous mutations (H63D or S65C) in the *HFE* gene have been reported to cause pathologic iron overload unless additional cofactors such as excess alcohol use or hepatitis C are present.[40,41] European Association for the Study of the Liver (EASL) guidelines recommend that genotyping for *HFE* should be performed in patients of European origin with biochemical evidence of iron overload (TSAT >45% and serum ferritin >200 ng/mL in women, and TSAT >50% and serum ferritin >300 ng/mL in men) regardless of symptoms.[42] If a patient is negative for *HFE* mutations, American College of Gastroenterology (ACG) guidelines recommend ruling out other causes of hyperferritinemia such as inflammatory conditions, chronic liver disease, and hemolytic anemias before pursuing workup for non-*HFE* hemochromatosis.[26] The most recent EASL guidelines in 2022 recommend that if a patient tests negative for *HFE,* testing should be performed for non-*HFE* mutations.[42] It is also recommended that first-degree relatives of homozygous heterozygotes should be tested for C282Y *HFE* mutation.[35,42,43]

Magnetic Resonance Imaging

Recent advances in magnetic resonance imaging (MRI) have revolutionized the diagnosis of iron overload.[44] MRI techniques for iron estimation have been developed based on the observation that iron-overloaded organs face faster signal decay.[45,46] Sarigianni and colleagues conducted a meta-analysis describing various MRI techniques for quantifying liver iron. Using hepatic iron concentration (HIC) as a reference standard, the authors reported the diagnostic accuracy of various MRI techniques for iron quantification to be variable.[47] Previous studies have also reported that HIC measured by biopsy is susceptible to large sampling errors, particularly in patients with moderate to severe iron overload or advanced fibrosis.[45,48]

There is now growing evidence that determination of HIC by MRI techniques may be more reliable and in some cases might be more accurate than liver biopsy

in assessing iron overload.[49] The different techniques used for the quantification of iron include T2 and T2* relaxometry/mapping and signal intensity ratio (SIR).[45,47] Relaxometry techniques measure the relaxation time by estimating signal decay at various echo times.[45] The results are presented as T2/T2* or R2/R2* (R2=1/T2, R2*=1/T2*). T2/T2* values have been reported to be inversely proportional to liver iron concentration (LIC), whereas R2/R2* values have been reported to be directly proportional to LIC.[45] A study by Wood and colleagues reported that MRI relaxometry is superior to liver biopsy for serial LIC measurements.[49] In another study by St Pierre and colleagues evaluating 233 patients with iron overload, the variance of differences between the 2 measurements of R2-MRI studies was lower than that from the liver biopsy.[50] Currently, FerriScan (Resonance Health) is the only MRI relaxometry technique approved by regulatory authorities for the diagnosis of iron overload.[51] Based on the evidence, both ACG and EASL guidelines recommend using MRI T2* to measure the HIC noninvasively.[26,42] For patients with juvenile hemochromatosis, cardiac MRI is also recommended, whereas for patients with hereditary aceruloplasminemia, brain MRI is recommended.[42]

Besides relaxometry and mapping, SIR has also been used to identify patients with iron overload. In this method, HIC is quantified by calculating the SIR between the liver and reference tissue.[45,52] Although it is easier to perform and provides a visual estimation of LIC, the SIR technique has been reported to be inferior to the relaxometry technique because of its limitations (eg, inability to account for fat effects, variability in the reference tissue, and tendency to overestimate LIC).[45,52] Currently, there is no consensus among experts regarding the data fitting procedures required to correct image noise and signal modulations from fat. Thus, the SIR method is not used routinely for iron measurement.

Splenic iron deposition can be used to differentiate HH from other causes of iron overload, such as hemolytic anemias and FD.[26] In patients with HH, a dark liver and a white spleen are noted, whereas in patients with other causes of iron overload, a dark liver and a dark spleen are noted.[46]

Update on Disease Staging

Currently, liver biopsy is used only for staging fibrosis in patients with *HFE* HH.[26,42] ACG guidelines recommend that liver biopsy be performed in C282Y homozygotes in certain conditions, such as a patient with a serum ferritin level of greater than 1000 ng/mL at diagnosis or concurrent risk factors for cirrhosis.[26,53,54] EASL guidelines recommend that a biopsy is also performed in patients with elevated liver enzymes or physical examination findings of hepatomegaly.[42] EASL guidelines do not recommend liver biopsy in patients with a clear diagnosis of cirrhosis based on physical examination findings or laboratory testing.[42] These patients should undergo regular hepatocellular cancer surveillance.

Determination of a hepatic iron index via liver biopsy was introduced as a means to distinguish homozygotes from heterozygotes or patients with alcohol-related liver disease.[55,56] A hepatic iron index of 1.9 or more can distinguish homozygous HH from heterozygotes or those with iron overload due to chronic liver disease.[55-57] However, after the availability of gene testing for *HFE* HH, the role of biochemical measurement of HIC content and calculation of a hepatic iron index has become less relevant.

Perl's Prussian blue stain is beneficial in identifying and characterizing the distribution of stored iron.[58] The pattern of iron deposition can also help differentiate between HH and other secondary iron overload disorders.[59-61] Iron is primarily located in periportal hepatocytes in patients with *HFE*-related HH (type 1), juvenile HH (type 2), and HH caused by mutations in the *TFR2* gene (type 3).[60,61] In contrast, in patients with FD and secondary iron overload disorders, the iron is found mainly in the Kupffer cells and little to no iron is present in the hepatocytes.[60,61]

With advances in technology as well as understanding of the disease, multiple noninvasive techniques have been developed to identify advanced fibrosis.[62,63] It is also pertinent to note that other clinical features of hemochromatosis may be beneficial in identifying patients with advanced hepatic fibrosis. In a study by Andersson and colleagues, the presence of advanced hepatic fibrosis was strongly associated with the development of arthritis, suggesting that patients with hemochromatosis arthritis should be screened for hepatic fibrosis.[64]

Noninvasive Markers of Fibrosis

The use of noninvasive blood tests and transient elastography has been well established in patients with other chronic liver conditions.[62,63,65,66] However, available data suggest that the threshold for diagnosing advanced fibrosis may be lower in hemochromatosis than in other disease states.[63,66] Ong and colleagues in their study of 134 newly diagnosed patients with hemochromatosis reported transient elastography as not diagnostic of advanced fibrosis. They suggested that a lower threshold might be beneficial in identifying patients with advanced fibrosis, as the degree of inflammation in *HFE* hemochromatosis-related liver injury is lower when compared with other diseases.[63]

ACG guidelines in 2019 did not routinely recommend transient elastography as a modality for assessing fibrosis.[26] However, more recent EASL guidelines recommend that patients with HH should be evaluated for liver

fibrosis using noninvasive techniques at diagnosis to guide appropriate treatment and follow-up.[42] EASL guidelines suggest that liver stiffness of 6.4 kPa or less effectively rules out advanced fibrosis in HH. Among patients with liver stiffness between 6.4 kPa and 12 kPa, EASL guidelines recommend performing a liver biopsy to rule out advanced fibrosis. For patients with liver stiffness greater than 12 kPa without elevated ferritin (>1000 ng/mL), transaminase levels greater than or equal to the upper limit of normal, or hepatomegaly, a liver biopsy is also recommended.[42] Further studies are needed to suggest if the cutoffs are adequate for diagnosing fibrosis.

Treatment

In patients with HH, treatment is initiated if they have an elevated serum ferritin (>300 ng/mL in men/postmenopausal women and >200 ng/mL in premenopausal women) and TSAT greater than 45%.[26,42]

Phlebotomy

Phlebotomy continues to be the first-line treatment for iron depletion in patients with HH.[26,35,42] Previous studies have reported that phlebotomy may improve fatigue, arthralgias, liver function tests, and regression of liver fibrosis and cirrhosis.[36,67,68] Studies have also reported that in patients with HH, morbidity and mortality are reduced if phlebotomy is initiated before the development of cirrhosis and diabetes.[67,69] There are 2 phases of treatment: induction and maintenance. The goal of the induction phase is to deplete iron stores with a target serum ferritin of less than 50 ng/mL. During the induction phase, phlebotomies can be weekly or biweekly.[26,42] During the maintenance phase, the goal is to avoid iron reaccumulation. Both ACG and EASL guidelines recommend a target serum ferritin of 50 to 100 ng/mL during maintenance.[26,42]

Erythrocytapheresis

Automated red blood cell exchange (erythrocytapheresis) is beneficial in the induction phase as fewer interventions are needed.[42,70] An open-label, randomized trial showed that erythrocytapheresis is associated with faster improvement in the ferritin level and decreased number of procedures compared with traditional phlebotomy.[70] Another study reported that erythrocytapheresis in the maintenance phase is related to the reduction in number of procedures required.[71] Erythrocytapheresis is associated with fewer hemodynamic changes than traditional phlebotomy and can benefit carefully selected patients.[71] Erythrocytapheresis has also been reported to be associated with improvement in the cognitive function for patients with hemochromatosis.[72] In a study by Ong and colleagues, of 104 patients with C282Y *HFE* hemochromatosis, erythrocytapheresis was associated with improvement in the Modified Fatigue Impact Scale, which was primarily driven by the improvement in the cognitive subcomponent of the score.[72]

Iron Chelation Therapy

Iron chelation therapy is recommended for patients in whom phlebotomy or erythrocytapheresis is not possible.[42] Chelation can be considered in patients who do not want phlebotomy, have anemia, or have life-threatening cardiac iron overload.[42] In these patients, the target ferritin is higher than targets in patients undergoing bloodletting procedures.[42] It is recommended that parenteral deferoxamine or oral deferasirox/deferiprone is only prescribed by physicians with clinical expertise because limited studies have assessed it as a safe alternative.[42,73-76]

New Therapies

Multiple hepcidin mimetics are currently under development as therapeutic tools for managing patients with HH.[77] Hepcidin mimetics might benefit patients with HH, as the underlying defect in HH is reduced hepcidin levels.[77] In murine models, hepcidin mimetics have been shown to prevent or limit iron accumulation in the liver and heart. These drugs also increased iron sequestration in the splenic macrophages.[78]

A phase 2 study of 16 patients with HH on a stable phlebotomy regimen of at least 0.25 phlebotomy per month for 6 months noted that subcutaneous rusfertide (PTG-300) reduced TSAT and serum iron levels. A corresponding decrease in the number of phlebotomies was also noted. The study also reported that rusfertide use was associated with the maintenance of LIC at prestudy levels with minimal use of phlebotomies.[79]

Another phase 2 clinical trial evaluating the effect of weekly LJPC-401 vs placebo in 70 adult patients with HH reported that the use of LJPC-401 resulted in a higher reduction in TSAT compared with the placebo group (-32.8% vs -2.5%).[80]

Other drugs that may be beneficial in the treatment of HH include FPN inhibitors.[81] A phase 1 study evaluating the effect of the FPN inhibitor VIT-2763 in healthy volunteers reported a decrease in serum iron levels after dosing.[81] Further studies assessing the impact of FPN inhibitors on patients with HH are needed.

Gene therapies are also currently under development for the management of HH. In the coming years, there may be gene therapies that may be curative for this disease. Recently, Rovai and colleagues provided a therapeutic gene approach based on targeted base editing of the C282Y mutation in the *HFE* gene in a mouse model and reported improvement in iron markers.[82] Further

human studies are needed to assess the safety and efficacy of this therapy. Considering that the majority of patients can be easily treated via phlebotomy, it is less likely that gene therapy will make its way into clinical practice for all patients with hemochromatosis.

Liver Transplantation

HH accounts for only 1% of the total liver transplants in the United States, despite a prevalence of 1 in 200 to 400 Whites.[83] This is because only a small proportion of C282Y homozygotes will develop significant iron overload to cause cirrhosis.[84] Studies have also suggested that additional comorbidities such as excess alcohol use, steatotic liver disease, and viral hepatitis are frequently present in C282Y homozygotes who require liver transplanation.[85] Studies prior to the implementation of the Model for End-Stage Liver Disease (MELD) score for liver transplantation reported that in patients undergoing transplantations, HH patients have worse outcomes than other groups.[86-89] A study in 2023 evaluating liver transplant outcomes between 2003 and 2019 reported excellent short- and long-term survival rates in patients with HH.[90] It was suggested that the adoption of the MELD score and other dynamic changes in the policies for candidate selection might have played a role in the improvement in survival and outcomes.[90] In previous studies, patients with hepatocellular carcinoma outside the current criteria for liver transplant were included, and this has been attributed as one of the causes for worse outcomes in the previous studies. It is also pertinent to note that the recent study is based on the *International Classification of Diseases, Tenth Revision* diagnostic codes to identify patients with HH and not based on genetic testing.[90] It has been previously reported that most patients with iron overload do not have HH and outcomes among patients with iron overload and HH are worse than those without HH.[88,91] It is very likely that some of the patients who were reported to have HH were not C282Y homozygotes or C282Y/H63D heterozygotes and this might have impacted results. Further studies with genetic test–confirmed HH in the current era are needed to assess whether the outcomes have really improved.

Conclusion

In recent years, there have been major advances in the understanding of the diagnosis and management of patients with genetic iron overload disorders. Currently, genetic testing for iron overload disorders is recommended for patients who have elevated TSAT with or without an elevated ferritin level. Patients with elevated ferritin levels but normal TSAT should have a workup to exclude secondary iron overload disorders and chronic liver conditions. The improvement in noninvasive techniques to quantify iron content and identify advanced fibrosis has decreased the need for liver biopsy in patients with HH. Finally, hepcidin mimetics can be a promising future therapeutic option and may reduce the need for phlebotomy in patients with HH.

Disclosures

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