Invited critical review

Hereditary hemochromatosis: Laboratory evaluation

Thomas P. Moyer⁎, W. Edward Highsmith b, Thomas C. Smyrk c, John B. Gross Jr. d

a Department of Laboratory Medicine & Pathology, Division of Clinical Biochemistry & Immunology, Mayo Clinic, Rochester, MN 55905, United States
b Department of Laboratory Medicine & Pathology, Division of Laboratory Genetics, Mayo Clinic, Rochester, MN 55905, United States
c Department of Laboratory Medicine & Pathology, Division of Anatomic Pathology, Mayo Clinic, Rochester, MN 55905, United States
d Department of Medicine, Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN 55905, United States

Abstract

The condition of hereditary hemochromatosis (HH) is caused by gene-dependent protein abnormalities involved in iron absorption, storage, or modulation of iron; these abnormalities result in iron overload. The clinical laboratory plays a significant role in case finding, diagnostic validation, and monitoring HH therapy. Elevated serum iron, transferrin saturation, and ferritin suggest HH, but results can also indicate other forms of hepatocyte injury such as alcoholic or viral hepatitis, or other inflammatory disorders involving the liver. In the context of elevated serum iron, transferrin saturation, and ferritin, and after ruling out secondary causes of iron overload, HFE gene evaluation is the preferred test to confirm the diagnosis of HH. However, 5% to 15% of patients with phenotypic HH do not have HFE gene mutations. In these cases, MRI evaluation or liver biopsy with iron quantification is indicated. The clinical role of hepcidin, the iron modulating protein, is undetermined at this time. Because hepcidin also plays a key role in antimicrobial and inflammatory activities, interpretation of hepcidin serum or urine concentration will require thorough understanding of its complex role in iron regulation.

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1. Overview

Diseases affecting the liver have profound consequences requiring early intervention. One common finding in liver disease is hemosiderosis, the condition of excessive iron accumulation, often described as iron overload. Liver is the first organ affected in iron-overload diseases. Iron catalyzes the formation of oxygen radicals that promote cell injury and activation of hepatic stellate cells, leading to fibrosis and, ultimately, cirrhosis. Increases in iron first appear in Kupffer cells and hepatocytes. This finding is commonly associated with sideroblastic anemia, excessive iron consumption, viral infection, chronic alcohol ingestion, or repeated transfusions. Persistent hemosiderosis with iron accumulation in biliary hepatocytes is typical of hereditary hemochromatosis (HH).

Hemochromatosis was first described in 1865 by Trousseau and later characterized and named by von Recklinghausen in 1889. The disease is characterized by an accelerated rate of intestinal iron absorption and storage, often leading to excess iron deposition in organs such as the liver, pancreas, heart, and joints. The clinical laboratory plays a significant role in case finding, diagnostic validation, and monitoring HH therapy.
absorption and progressive iron deposition in various tissues that is typically expressed in the third to fifth decades of life; the most severe variants of the disease may be expressed in children. Because of the severe sequelae of this disease if left untreated, and recognizing that treatment is relatively simple, early diagnosis is important before signs or symptoms appear.

In a review, Franchini and Veneri [1] defined several variants of hereditary hemochromatosis (HH).

• Hereditary hemochromatosis type I (OMIM 235200) is the classical HFE-related disorder associated with mutations in the HFE gene expressed as HFE protein variants C282Y and H63D. This is an autosomal recessive disease with estimated prevalence in the population of 2 in 1000 in Caucasians, with lower incidence in other races. This gene is at chromosome location 6p21.3; the majority of hemochromatosis patients have this type.

• Hereditary hemochromatosis type IIa (OMIM 602390), also called juvenile hemochromatosis (JH), is a rare, more severe form of the disease that occurs before age 30, caused by mutations in the hemojuvelin gene (HJV) located on chromosome 1q21.

• Hereditary hemochromatosis type IIb (OMIM 606464) is even more severe than type IIa, caused by mutations in the HAMP gene located on chromosome 19q13 coding for the protein/hormone hepcidin.

• Hereditary hemochromatosis type 3 (OMIM 606250) is an autosomal recessive disease caused by mutations in the transferrin receptor 2 gene (TFR2) located on chromosome 7q22 coding for the protein/hormone hepcidin.

• Hereditary hemochromatosis type 4 (OMIM 606069) is an autosomal dominant disease caused by mutations in the SLC40A1 gene coding for the protein/hormone hepcidin-resistant form (C326S) of the metal transport protein ferroportin.

• Hyperferritinemia-cataract syndrome is a rare autosomal dominant disease caused by mutations in the ferritin L-subunit gene located on chromosome 19q13 coding for the protein iron-responsive element (IRE) that facilitates high serum ferritin without iron overload.

Serum iron concentration and transferrin saturation are commonly used clinical tests to screen for HH. If the serum transferrin saturation is elevated, serum ferritin evaluation is recommended. Elevated serum iron, transferrin saturation, and ferritin, indicating increased iron stores, are commonly associated with HH, but also with other forms of hepatocyte injury such as alcoholic or viral hepatitis, or systemic inflammatory disorders. HFE gene evaluation is the next test to be considered in the diagnostic algorithm for HH, or for predictive testing of individuals who have a family history of HH [2, 3]. If HFE gene evaluation is negative, and no other obvious cause for increased iron stores is evident, liver biopsy histologic evaluation and iron quantification may be performed. Iron accumulation in biliary hepatocytes and increased iron concentration indicate HH, which is responsive to regular phlebotomy therapy (Fig. 1).

2. Normal iron metabolism

A typical human adult absorbs 1–2 mg of iron per day from the diet. Iron is taken up from the lumen of the intestine by the duodenal villus cell through a process involving Ferrireductase (DcytB) and Divalent Metal Ion Transporter (DMT1, SLC11A2), both membrane-bound proteins. Apical membrane-bound DcytB converts absorbed Fe⁺³ to Fe⁺². Apical membrane-bound DMT1 transports Fe⁺² from the lumen of the intestine into the villus cell. Cellular Fe⁺² is subsequently transported from the cell into the blood via a basal membrane-bound iron transporter, ferroportin (SLCA40A1). In the blood, Fe⁺² is converted to Fe⁺³ by the villus cell basal membrane-bound protein, hephaestin. In the Fe⁺³ state, iron binds avidly to apo-transferrin to form transferrin, the primary form of protein bound iron circulating in the blood serum [4, 5] (Fig. 2).

Downstream from the duodenal villus cell, the duodenal crypt cell re-absorbs iron through a process involving a complex comprised of circulating beta-2-microglobulin with crypt cell membrane-bound...
transferrin receptor (TfR2) and membrane-bound HFE protein, converting Fe$^{3+}$ to Fe$^{2+}$ during endocytosis. The duodenal crypt cell is one of the principle iron storage sites in the body [4]. Feder et al. [6] demonstrated a conformation-dependent interaction between TfR2, beta-2-microglobulin, and HFE protein required to facilitate the endocytic process; transport was reduced when HFE protein alteration (C282Y) interfered with beta-2-microglobulin–HFE protein interaction. HFE and TfR2 also interact; HFE binding stabilizes TfR2 [7, 8].

Under the direct feedback inhibition via the exocrine protein hepcidin (Hamp, LEAP1), and modulated by an interaction with Janus kinase 2 (Jak2), duodenal crypt cell membrane-bound ferroportin reintroduces Fe$^{2+}$ into the blood. Similar to the case for villus cell release, when Fe$^{3+}$ is released into the blood it is converted to Fe$^{2+}$ by crypt cell membrane-bound hephaestin, a copper oxidase homologous to ceruloplasmin. The Fe$^{3+}$ binds to apotransferrin and forms transferrin which circulates in the blood serum and serves to transport iron to other organs [9].

Transferrin circulating in blood serum presents iron to the liver and other organs where it is absorbed to support cytochrome-dependent electron transport and other oxidative processes, and to the bone marrow to support the formation of erythrocytes. During a typical day, an adult human integrates 20–25 mg of iron into erythrocytes via a TR1 modulated process. Likewise, during a typical day, phagocytes remove erythrocytes containing 20–23 mg of iron, reprocessing the iron in a manner similar to the duodenal crypt cell (but involving TR1 instead of TR2), retaining iron in the form of transferrin to support iron homeostasis. Conservation of iron has been a critical attribute in mammalians to preserve the species. Iron elimination is minimal, achieved predominantly via fecal elimination as well as cell shedding, sweat, menses, and a trivial amount excreted by the kidney.

Blood carrying transferrin bound iron enters the liver via portal circulation and moves through the liver toward the central vein; as blood is transported through the liver it is in contact with hepatocytes. Through a process similar to the duodenal crypt cell, a complex comprised of circulating beta-2-microglobulin, hepatocyte membrane bound TfR2, and membrane-bound HFE, iron is taken up via endocytosis and simultaneously converted from Fe$^{3+}$ to Fe$^{2+}$. Within the hepatocyte, several processes occur that are dependent upon the concentration of Fe$^{2+}$ in the endocyte: 1) Interleukin (IL1 and IL6) mediated nuclear transcription and translation occurs to stimulate the synthesis of hepcidin and hemosiderin [9]; 2) The concentration of Fe$^{2+}$ mediates nuclear transcription and translation occurs to stimulate the synthesis of apotransferrin and ceruloplasmin; 3) Passive effusion of Fe$^{2+}$ from the endocytic vesicles to the cytoplasm occurs where it binds to ferritin and is engulfed in lysosomes to become hemosiderin; and 4) Cytosolic Fe$^{2+}$ is actively transported out of the hepatocyte by ferroportin into the blood to recirculate, and into the biliary tract to be excreted in the feces [7, 10] (Fig. 3).

3. Defects in hereditary hemochromatosis

In HH, excess iron enters the bloodstream beyond what is required for erythropoiesis. While erythropoiesis is unimpaired, in the absence of other metabolic disorders, this unregulated iron traffic causes increased saturation of transferrin and iron accumulation in parenchymal cells; sustained iron overload leads to cirrhosis, hypogonadism, diabetes, cardiomyopathy, arthropathy, or skin pigmentation. In HH, macrophages release more iron than in wild-type individuals. Increased serum iron is mainly due to increased release from enterocytes into blood rather than increased uptake from the intestinal lumen. These macrophage and enterocyte abnormalities are due to unrestricted ferroportin–mediated iron export that arises from a deficiency of hepcidin [10].

Rapid and massive influx of iron into the plasma can cause a severe, early-onset organ disorder called juvenile hemochromatosis (type 1a) that includes heart failure and endocrine insufficiency. The cardiac and endocrine systems are particularly susceptible to rapid iron loading, probably because their cells have more mitochondria and fewer antioxidants than liver cells. The rapid buildup of iron in these cells causes oxidative stress, limiting high-energy electrons to support mitochondrial respiration. In contrast, gradual iron loading leads to a milder phenotype with later onset of symptoms (type I). Intermediate phenotypes have also been described. The hemochromatosis phenotype is determined primarily by the rate and magnitude of the circulatory iron overload, which depends on the specific protein that is altered and its interaction with hepcidin. The common denominator for these genetic mutations is hepcidin deficiency [10-12].

In organs such as liver, pancreas, heart, and gonads, the iron content of parenchymal cells increases progressively and finally exceeds the storage capacity of the intracellular iron storage protein ferritin. Without treatment, circulatory iron excess eventually affects organs, particularly parenchymal cells, leading to the production of...
highly reactive oxygen species that damage intracellular structures [13].

4. Role of copper

It is imperative to understand that copper plays a large role in iron homeostasis. Iron and copper are highly interactive physiologically at points of absorption in the intestinal tract (enterocytes), management in the liver (hepatocytes), in cells involved in iron recycling and storage (reticuloendothelial macrophages), and erythroid cells that utilize the vast majority of iron for hemoglobin production. Dietary iron and copper are absorbed in the upper small bowel. Iron absorption is controlled by the liver-derived peptide hormone hepcidin which inhibits ferroportin-mediated iron release from intestinal cells. Iron copper uptake in the gut are regulated by hephaestin, by dietary copper, and by copper ATPase. There are no regulated excretory mechanisms for iron, whereas copper is excreted in the bile (mediated by ATP7B). Iron secreted from the liver is bound to ceruloplasmin, albumin, or αβ2-macroglobulin. Most iron is delivered to the bone marrow for hemoglobin production. The major iron oxidation protein, ceruloplasmin, requires copper as a cofactor, and the major membrane bound iron oxidizing protein hepahesiain requires multiple copper ions for activity. Bone marrow iron utilization is copper dependent; during copper deficiency, hemoglobin production is inefficient despite normal serum iron levels [14].

For more detailed review of the molecular biology and cellular basis of iron overload, readers are referred to excellent reviews on these topics by Camaschella [12], Yen et al. [15], Adams and Barton [16], Chua et al. [17], Knovich et al. [18], Galaris and Pantopoulos [13], Gray et al. [19], Collins et al. [14], Hentze et al. [9], Horvathova et al. [20], and Ramm and Ruddell [21].

5. Iron, transferrin saturation, and ferritin

A clinical practice guideline describing standard case finding practice for HH published by the American College of Physicians [22] recommends serum ferritin and transferrin saturation tests for HH screening; a literature review by Schmitt et al. [23] substantiates these recommendations. If testing is performed, serum ferritin >200 mcg/L in women or >300 mcg/L in men, and transferrin saturation >45% in women or >55% in men, are the criteria for case-finding. Ferritin values >1000 mcg/L are frequently associated with cirrhosis. There were no recommendations regarding screening for the purpose of risk-stratifying patients with associated conditions such as type 2 diabetes, cardiac arrhythmias and cardiomyopathies, liver failure, hepatomegaly, cirrhosis, elevated liver enzyme levels, hepatocellular carcinoma, arthritis, hypogonadism, or changes in skin pigmentation [24].

The HEmochromatosis and IRon overload Screening (HEIRS) study [25-27] evaluated the prevalence, genetic, and environmental determinants, and potential clinical, personal, and societal impact of hemochromatosis and iron overload in a multiethnic, primary care-based sample of 101,168 adults (44% Caucasians, 27% African-Americans, 14% Asians/Pacific Islanders, 13% Hispanics, 2% others; 63% female and 37% male) over a 5-year period. Because transferrin saturation was considered the best single screening test for HH, a combination of transferrin saturation and serum ferritin values derived from the third National Health and Nutrition Examination Survey [28] were used to define thresholds to identify the participants to be examined and genotyped for the C282Y and H63D variants. HEIRS data indicate that transferrin saturation >50% and ferritin >300 mcg/L had odds ratios for HH from 120 to 1704 (very high odd ratios). Transferrin saturation was found to be the best single test for screening.

Transferrin saturation is widely considered the preferred screening test for HH. Unsaturated iron-binding capacity (>150 mcml/L for women, >125 mcml/L for men) was reported to have similar performance [29]. Adams et al. [27] noted that within-person biological variability of transferrin saturation and unsaturated iron-binding capacity limited their usefulness as an initial screening test for C282Y homozygotes. This, in part, may reflect the difference between HFE-related HH and phenotypic disease; subjects with iron overload but negative for C282Y have a different variant of HH.

A practice guideline published by the European Association for the Study of the Liver (EASL) [30] notes that validation studies assessing body iron stores achieved by phlebotomy indicate that serum ferritin is a highly sensitive test for HH case finding and recommend that serum ferritin is the preferred biochemical surrogate for iron overload [31]. Normal serum ferritin concentration rules out iron overload. However, ferritin has low specificity for HH; elevated values can be the result of inflammatory, metabolic, and neoplastic conditions such as diabetes mellitus, alcohol consumption, viral infection, and hepatocellular or other cell necrosis or neoplasms. EASL [30] recommends that serum iron concentration and transferrin saturation do not quantitatively reflect body iron stores and should not be used as surrogate markers of tissue iron overload.

The frequency of elevated serum ferritin in the primary-care setting reflecting increased iron stores is not known. HEIRS data in...
HFE C282Y homozygotes regardless of iron measures, suggest a prevalence in the Caucasian population of C282Y homozygotes with serum ferritin >900 mcg/L of 20 per 10,000 men and 4 per 10,000 women; these parameters were predictive of iron stores >4 g in men and >2 g in women. The estimated prevalence per 10,000 in non-C282Y homozygotes with transferrin saturation >45% in women or >50% in men and with serum ferritin >900 mcg/L was 7 among Caucasians, 13 among Hispanics, 20 among African Americans, and 38 among Asians and Pacific Islanders; these parameters were predictive of iron stores >2 g but <4 g. Serum ferritin >900 mcg/L after initial elevations of both serum ferritin and transferrin saturation was predictive of increased iron stores in multiple ethnic populations regardless of HFE genotype [32].

6. Genetics

The most common form of HH is described by variants in the HFE gene located on the short arm of chromosome 6 that encodes a 343-amino acid protein; this protein is an HLA Class I molecule. Two point mutations occur in the HFE gene at exon 4, 845G-A, and exon 2, 187C-G, that cause variants in the HFE protein designated C282Y and H63D. When there is a clinical suspicion of HH, approximately 85% of these patients are found to be homozygous for the C282Y mutation, or compound heterozygous for C282Y and H63. In the US Caucasian population, approximately one in 200 persons is homozygous for the C282Y mutation, while one in 10 is a heterozygous carrier. The greatest risk for iron overload exists in those who are homozygous for the C282Y mutation. The penetrance of two copies of the C282Y mutation for biochemical evidence of iron overload is quite high (but not 100%); however, the penetrance for actual clinical disease is very low. A study of a large cohort of patients attending a health maintenance clinic [31] showed that the fraction of C282Y homozygous with clinical symptoms was less than 1%. Approximately 3% to 7% of the US Caucasian population are C282Y/H63D compound heterozygotes and 3% to 5% are H63D homozygotes [31,33-36]. Only a small percentage of all C282Y/H63D compound heterozygotes or H63D homozygotes will develop iron overload, and the condition is usually mild. One copy of the H63D mutation is present in 20% of the normal population and by itself does not increase the risk for developing iron overload [37].

The presence of the C282Y form of HFE protein disrupts cellular trafficking, blocking its localization at the cell membrane, and preventing interaction with beta-2-microglobulin due to disruption of a critical intramolecular disulfide bond which requires the cysteine at amino acid 282 [6]. The same study found few, if any, changes in cellular localization or beta-2-microglobulin interaction of H63D bearing proteins as compared to the wild type.

The HFE gene test is useful for screening adult blood relatives of a C282Y homozygous proband. Screening blood relatives is crucial because 25% of siblings and 2–3% of children of a proband will have HH. In addition, HFE gene testing is useful in helping to resolve ambiguous cases, such as mild iron overload associated with hepatitides C infections, alcoholic liver disease, or other causes of end-stage liver disease. Genetic anomalies can be confounding; for example, a previous bone marrow transplant from an allogeneic donor may cause false-negative genetic testing. HFE gene testing has replaced the more expensive HLA typing previously used to screen siblings. Because of concerns about the possibility of insurance, employment, or other discrimination based on HFE test results, HFE gene testing usually is not recommended for anyone younger than 18 years old. There is no test that can predict whether a C282Y homozygote will develop clinical symptoms.

Since the discovery of the C282Y and H63D mutations, approximately 18 additional HFE gene mutations have been reported. In general, most of these additional mutations are uncommon and rarely of clinical significance. S65C is caused by a mutation in the HFE gene at exon 2, 193A-T that occurs in 1% to 2% of the normal population. There are rare reports of iron overload occurring in patients with 1 copy of the S65C, usually in association with C282Y [38]. In a retrospective analysis of HFE tests done on patients with demonstrated iron overload (as defined by the removal of >5 g of iron by therapeutic phlebotomy) seen in a specialty hemochromatosis service at Mayo Clinic, the only individuals noted to carry the S65C mutation were also heterozygous for C282Y.

Shaheen et al. [42] predicted that 15% of patients with iron overload typical of HH would not have the C282Y mutation, consistent with the findings of Zoller and Cox [39], Walsh et al. [37], and Whitlock et al. [36]. In most studies, 5% to 15% of subjects with clinically significant iron overload do not have HFE gene mutations. Therefore, a negative HFE gene test does not exclude the possibility of a subject developing phenotypic disease.

Unfortunately, confusion or misunderstanding in clinical practice has developed related to the use of the terms hemochromatosis, hereditary hemochromatosis, HFE-related hemochromatosis, and phenotypic hemochromatosis. Some studies appear to equate C282Y homozygosity to HH. Focused statements describing studies targeting C282Y, such as “It is now largely accepted that the p.C282Y/p.C282Y genotype is necessary for the development of HFE haemochromatosis”, [40] or “Transferrin saturation has high biological variability and relatively low sensitivity to detect HFE C282Y homozygotes” [41] confuse clinicians. C282Y homozygosity, while the descriptor for HFE hemochromatosis, is not required for HH. It has been shown repeatedly that approximately 15% of Caucasians with iron overload typical of HH are not C282Y homozygotes. In the unique clinicopathologic set of iron overload syndromes, the disorder related to C282Y homozygous mutation of the HFE is the most common form of HH, but it is not the sole form [19, 42, 43].

In a meta analysis of nine independent studies, Jin et al. [44] reported a modest association of C282Y with HH, but a significant odds ratio of 2.08–7.92 for the C282Y allele is associated with hepatocellular carcinoma in patients with alcoholic liver disease, suggesting individuals homozygous for C282Y have a greater susceptibility to alcohol-induced liver disease and hepatocellular carcinoma.

7. Hepatic iron

Prior to the availability of the HFE gene test, a liver biopsy was often necessary to confirm the diagnosis of HH; since the implementation of genetic testing for HH, the need for hepatic evaluation has been greatly reduced. Indications for liver biopsy in a person known to be homozygous for the C282Y include: assessment of disease stage (fibrosis), assessment of coexistent liver disease, and identification of neoplastic or neoplastic lesions. Patients older than 40 years, with serum ferritin levels >1000 mcg/L, increased transaminase activity, and hepatomegaly are at risk for hepatic fibrosis and cirrhosis and could be candidates for biopsy [10]. Coexistent fatty liver disease is a cofactor in liver injury in HH, and histologic documentation of that pathology will help direct clinical management. Early detection of hepatocellular carcinoma associated with HH might also be detected [44-46].

Hepatic iron deposition has a characteristic pattern in HH, beginning in perportal (zone 1) hepatocytes and extending progressively to involve all zones of the liver. In the most severe cases, iron will also be deposited in biliary epithelium and the reticuloendothelial system (predominantly Kupffer cells). Conversely, iron deposition in secondary hemosiderosis occurs primarily in Kupffer cells and only involves hepatocytes when present in large quantities. Perls' stain will demonstrate the characteristic Prussian-blue granules at the bile canalicular pole in HH. There are a number of semi-quantitative methods for scoring iron overload, based on the size and distribution of hemosiderin granules. Schauer and Lefkowitch [47] proposed a simple scheme in which grade 1 represents minimal hemosiderosis,
grade 4 massive deposits with loss of the usual zonal gradient, and grades 2 and 3 intermediate amounts. While liver biopsy is not necessary for the diagnosis of HH in patients known to be homozygous for C282Y mutations, hepatic hemosiderosis of grade 2 or more, in the pattern described above, should prompt evaluation for HH (Fig. 4).

Hepatic iron may be quantified by spectrometric methods. Hepatic iron concentration >3000 mcg/g dry wt indicates iron overload [48-50]. Patients with hepatic cirrhosis due to hepatitis C or alcohol, and those with fibrosis due to sickle cell disease (without transfusion) with significant fibrosis are likely to have HIC at or just above the top end of normal (<2400 mcg/g dry wt for men, <1600 mcg/g dry wt for women) [40, 51]. Mildly elevated HIC (3000 > HIC < 5000 mcg/g dry wt) is most frequently associated with secondary hemosiderosis or sideroblastic anemia. An HIC > 10,000 mcg/g dry wt is often associated with cirrhosis and is seen only in HH, or chronic transfusion-related iron overload as occasionally seen in treatment of thalassemia or sickle cell disease. While mildly elevated HIC is common in patients with heterozygous for one HFE gene mutation, it is not known whether asymptomatic HH patients with mildly elevated HIC represent early stage homozygous HH [18, 52-54].

Iron accumulates in liver with age. Halliday’s group [55] developed the hepatic iron index (HII), the expression of hepatic iron concentration in micromoles/gram dry weight liver adjusted for the patient’s age in years; this normalizes hepatic iron concentration for age. The HII originally was intended to distinguish HH homozygotes from heterozygotes and those with alcoholic liver disease. In the initial study, all patients with phenotypic HH had an HII > 1.9, whereas all patients with alcohol or HCV-related liver disease had a HII < 1.9. Patients with heterozygous HH often have HII ranging from 1.0 to 1.9. Patients with hepatitis and alcoholic cirrhosis usually have HII < 1.0, although a small percentage of patients with alcoholic cirrhosis have HII in the range of 1.0 to 1.9. Patients with homozygous hemochromatosis have HII > 1.9. Patients with hemochromatosis who have been successfully treated with phlebotomy will have HII < 1.0. This finding was validated by Ludwig et al. [48, 49], Bonkovsky et al. [56] and Sajjad et al. [57].

For many years, a HII > 1.9 was considered diagnostic of HH, whereas HII values < 1.9 were considered inconsistent with the diagnosis. More recent studies have demonstrated that a HII > 1.9 is not diagnostic of HH because patients with transfusion-related iron overload are likely to have an HII > 1.9. Bassett [58] described the role of HII in review, pointing out an “over reliance on genetic testing instead of investigations of iron overload”… “it is essential to confirm a diagnosis of hemochromatosis on the basis of increased body iron stores”… “liver biopsy remains the best method of confirming this”, because HFE-related HH is increasingly diagnosed at an earlier stage by genetic testing, some patients with HFE-related HH who have been successfully treated will have a HII < 1.9 [59, 60]. There are no studies describing whether homozygous C282Y patient with HII < 1.9 go on to develop phenotypic disease.

Although HII is the gold standard for assessment of iron overload [18], interest in non-invasive imaging techniques for evaluation of HIC has evolved. Superconducting quantum interference device (SQUID) biomagnetometry [30, 39, 60] and magnetic resonance imaging (MRI) [30, 50] are being used in clinical practice. The SQUID technique is reported to correlate well with HIC when comparing biopsy tissue, but was not been independently validated in HH patients [30]. MRI utilizing magnetic gradient echo (T2*) is more extensively used and allows repeated measurements; however, MRI results do not correlate strongly with HII (r ~0.75). MRI studies have tended to focus on myocardial evaluation where non-invasive techniques provide significant advantage [18, 30, 39, 50].

The conundrum here is that HH represents a continuously evolving state of iron overload. In a patient with negative HFE gene testing, iron overload for no other obvious reason, and family history of liver disease, evaluation of HIC and HII should be considered. Liver biopsy evaluation is appropriate when:

- Serum iron is > 175 mcg/dL,
- Transferrin saturation is > 55% in males or > 45% in females,
- Ferritin is > 400 ng/mL,
- HFE gene test is negative for known HFE variant, and
- No other cause for iron overload can be identified.

8. Hepcidin

In 2006, Pietrangelo [61] noted that “...all known hemochromatosis in common the same metabolic abnormality: the genetically determined failure to prevent unneeded iron from entering the circulatory pool. Inappropriate levels of hepcidin, the iron hormone, appear now as the central pathogenic event in all forms of hemochromatosis...”. The common phenotypic characteristics of HH all appear to be related to the deficient synthesis or reduced activity of hepcidin. As a result, hepcidin has been identified as a hormone and the disease as an endocrine disease. This protein inhibits the entry of iron into the bloodstream. All known genetic variants associated with HH (HFE, TFR2, and HJV), as well as HAMP mutations that code for the protein herceptin, affect the interaction of hepcidin with the transmembrane iron export protein ferroportin [10].

Hepcidin, first reported in 2000 by Krause et al. [62] and Park et al. [63], is detectable in biological fluids. Undetectable or very low urine hepcidin concentrations have been reported in patients with HH [64]; serum assays have also been developed [65]. Investigators are examining pro-hepcidin to further understand the role of this important peptide [66]. Biological specimen evaluation of hepcidin and prohepcidin concentrations in a cohort of affected families have been reported [67].

While its importance in iron status is clear, use of hepcidin in the clinical evaluation of HH will be confounded by the observation that hepcidin is also involved in the inflammatory pathway. Hepcidin is an antimicrobial peptide; inflammation stimulates the release of interleukins IL1 and IL6 which result in induction of hepcidin; in
severe cases this action may lead to anemia [68]. Therefore, introduction of hepcidin evaluation into clinical practice will require considerable study and understanding.

9. Treatment

Iron can be effectively, efficiently, and safely removed by phlebotomy [69]. Therapeutic phlebotomy is initiated by withdrawing blood at a rate of 500 mL per week until ferritin is <50 mcg/L. Patients with SCL40A1 (ferroportin deficiency) undergoing frequent phlebotomy may be at risk for anemia; all phlebotomy events should be monitored by careful follow-up of hemoglobin. Phlebotomy therapy is tailored to each patient to achieve a maintenance ferritin <50 mcg/L; this may involve several phlebotomies per year. Phlebotomies achieving ferritin well below 50 mcg/L ferritin levels may increase iron absorption in C282Y homozygotes due to low hepcidin concentration [70].

The oral iron chelator deferiprone (Ferriprox) was not found to be suitable for genetic iron overload due to risk of agranulocytosis. The new oral iron chelator deferasirox (Exjade) is not registered for HH, and should only be used in exceptional cases, since conventional phlebotomies have much lower side effects [70].

10. Summary

A typical human adult absorbs 1–2 mg of iron per day from the diet. Iron is taken up in the lumen of the intestine by the duodenal villus cell and transported into the circulatory system. In blood, iron circulates bound to the protein transferrin. Downstream from the duodenal villus cell, the duodenal crypt cell re-absorbs iron from the circulatory system; this cell serves as one of the primary body storage sites for iron. When iron is needed, decreased circulating concentration of hepcidin allows the crypt cell to re-introduce iron into the blood. Liver hepatocytes also store iron and serve as the primary sensory cells modulating body iron stores; the iron modulating hormone hepcidin is synthesized and released by hepatocytes. The condition of HH is caused by gene-dependent protein abnormalities involved in iron absorption, storage, or modulation of iron; these abnormalities result in iron overload.

The clinical laboratory plays a significant role in case finding, diagnostic validation, and monitoring HH therapy. Serum iron concentration and transferrin saturation are commonly used clinical tests to screen for HH. If the serum iron concentration is >175 mcg/dL and transferrin saturation >45% in women or >55% in men, analysis of serum ferritin concentration should be performed. Elevated serum iron, transferrin saturation, and ferritin >200 ng/mL in women or >300 ng/mL in men suggest HH, but results can also indicate other forms of hepatocyte injury such as alcoholic or viral hepatitis, or other inflammatory disorders involving the liver. Serum ferritin >900 mcg/L predicts increased iron stores in multiple ethnic populations in all HH genotype. Current clinical practice guideline describing standard case finding practice for HH [22] recommends serum transferrin saturation and ferritin tests for HH case finding.

The frequency of elevated serum ferritin in the primary-care setting reflecting increased iron stores is not known. The HEIRS study estimated the incidence of homozygous HFE-related HH with serum ferritin >900 mcg/L at 20 per 10,000 men and 4 per 10,000. The estimated prevalence in 10,000 by race with serum ferritin >900 mcg/L in non-HFE-related homozygous HH patients is 7 among Caucasians, 13 among Hispanics, 20 among African Americans, and 38 among Asians and Pacific Islanders.

In the context of elevated serum iron, transferrin saturation, and ferritin >200 ng/mL in women or >300 ng/mL in men, HFE gene evaluation is recommended to confirm the diagnosis of HH, or for predictive testing of individuals who have a family history of HH. Iron overload with homozygous variant C282Y in the HFE gene is diagnostic for HH. However, patients found to have homozygous mutations in HFE gene without iron overload (found in population-based studies) may not require treatment [16].

Approximately 3% to 7% of the US Caucasian population are C282Y/H63D compound heterozygotes and 3% to 5% are H63D homozygotes. The presence of heterozygous H63D mutation is 20% of the population and by itself does not increase the risk for developing iron overload. A small percentage of all C282Y/H63D compound heterozygotes or H63D homozygotes develop iron overload, and the condition is usually mild.

The HFE gene test is useful for screening adult blood relatives of a C282Y homozygous proband. Screening blood relatives is crucial because 25% of siblings and 5% of children of a proband will have HH. HFE gene testing has replaced the more expensive HLA typing previously used to screen siblings. In addition, HFE gene testing is useful in helping to resolve ambiguous cases, such as mild iron overload associated with hepatitis C infections, alcoholic liver disease, or other causes of end-stage liver disease.

Since the discovery of the C282Y and H63D mutations, approximately 18 additional HFE gene mutations have been reported. There are reports of iron overload occurring in patients with 1 copy of the S65C, usually in association with C282Y. Results for S65C are usually reported for patients who also have a C282Y mutation. 5% to 15% of patients with HH with clinically significant iron overload do not have HFE gene mutations. Therefore, a negative HFE gene test does not exclude the possibility of a subject developing phenotypic disease.

Implementation of genetic testing for HFE gene mutations has changed clinical practice. Prior to genetic testing, liver biopsies were required to make the diagnoses; this is no longer the case. MRI evaluation, a noninvasive technique, is used in many academic medical centers to follow disease progression. However, in patients with iron overload and negative genetic studies for HFE gene variants, when other causes of iron overload such as viral infection, chronic alcohol use, or transfusion-related iron overload have been ruled out, evaluation of HIC via liver biopsy may be indicated. Hepatic iron concentration >3000 mcg/g dry wt indicates iron overload. Patients with hepatic cirrhosis due to hepatitis C or sickle cell disease (without transfusion) with significant fibrosis are likely to have HIC at or just above the top end of normal (2400 mcg/g dry wt for men, <1600 mcg/g dry wt for women). Mildly elevated HIC (3000 > HIC <5000 mcg/g dry wt) is associated with secondary hemosiderosis or sideroblastic anemia. While mildly elevated HIC is common in patients with heterozygous HH, it is not known whether asymptomatic HH patients with mildly elevated HIC represent early stage homozygous HH. An HIC >10,000 mcg/g dry wt is virtually always associated with cirrhosis and fibrosis and is seen in HH or chronic transfusion-related iron overload as occasionally seen in treatment of thalassemia or sickle cell disease.

The HIL, used infrequently since the advent of HFE testing, is the reference test to define iron overload. The normal range for HIL is <1.0, iron overload with 1–2 HIL requires careful evaluation for sickle cell disease, HCV, or chronic alcohol use, and HIL >2 in the absence of transfusion-related iron overload (thalassemia) is considered diagnostic for HH.

The clinical role of hepcidin evaluation is undetermined at this time. While decreased circulating hepcidin concentrations are found in HH, hepcidin may also be decreased in inflammation and infection. Interpretation of hepcidin concentration will require thorough understanding of its complex role in iron regulation.

References


