Classification and diagnosis of iron overload

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Abstract

Background and Objective. Iron overload is the result of many disorders and could lead to the development of organ damage and increased mortality. The recent description of new conditions associated with iron overload and the identification of the genetic defect of hereditary hemochromatosis prompted us to review this subject and to redefine the diagnostic criteria of iron overload disorders.

Evidence and Information sources. The material examined in the present review includes articles published in the journals covered by the Science Citation Index® and Medline®. The author has been working in the field of iron overload diseases for several years and has contributed ten of the papers cited in the references.

State of the art and Perspectives. Iron overload can be classified on the basis of different criteria: route of access of iron within the organism, predominant tissue site of iron accumulation and cause of the overload. Excess iron can gain access by the enteral route, the parenteral route, and placental route during fetal life. The different distribution of iron within parenchymal or reticuloendothelial storage areas indicates different pathogenetic mechanisms of iron accumulation and has relevant implications in terms of organ damage and prognosis of the patients. Iron overload may be either primary, resulting from a deregulation of intestinal iron absorption as in hemochromatosis or secondary to other congenital or acquired conditions. Diagnosis of iron overload can be suspected on the basis of clinical data, high transferrin saturation and/or serum ferritin values. However, several hyperferritinemic conditions are not related to iron overload, but may imply severe disorders (inflammations, neoplasia) or a deregulation of ferritin synthesis (hereditary hyperferritinemia-cataract syndrome), and iron overload secondary to aceruloplasminemia, and the recently described dysmetabolic-associated liver iron overload syndrome, are characterized by low or normal transferrin saturation levels. Liver biopsy is still very useful in the diagnostic approach to iron overload disorders, by defining the amount and the distribution of iron within the liver. The analysis of HFE gene mutations (C282Y and H63D) is a simple and strong tool in the diagnostic work out of iron overload conditions.

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Key words: iron overload

Iron overload is the result of many disorders and could lead to the development of organ damage and increased mortality. The recent description of new conditions associated with iron overload and the identification of the genetic defect of hereditary hemochromatosis prompted us to review this subject and to redefine the diagnostic criteria of iron overload disorders.

Classification of iron overload

Iron overload is the result of many disorders and could lead per se to the development of organ damage and increased mortality. In humans total body iron stores is maintained normally within the range of 200-1500 mg (in men, the normal concentration of iron in the storage pool is 13 mg/Kg and in women 5 mg/kg) by adequate adjustment of intestinal iron absorption, since no excretory mechanisms exist. In normal individuals, feedback mechanisms inhibit iron absorption as storage iron increases. Each condition that induces an increased net entry of iron within the body inevitably leads to iron overload. Iron overload could be defined as an increase of storage iron, regardless of the presence or absence of tissue damage. It can be classified on the basis of different criteria: route of access of iron within the organism, predominant tissue site of iron accumulation and cause of the overload.

Route of access of iron

Excess iron can gain access to the organism in three ways: the enteral route, through absorption of dietary heme and non-heme iron, the parenteral route, through transfusions or injections of iron-containing compounds, and placental route during fetal life (Table 1).

Enteral route

Hereditary hemochromatosis (HHC) leads to absorption of excess dietary iron. Besides the recent discovery of the genetic defect in HHC, the pathogenic mechanism leading to inappropriately high iron absorption in this disorder is still undefined. The excess of daily iron uptake is not large, but over time, due to the absence of iron excretory pathways in humans, substantial body overload develops. In juvenile hemochromatosis, iron absorption is also increased, but the genetic as well as the biochemical defect of this kind of hemochromatosis is still
unknown. Ineffective erythropoiesis is associated with increased uptake of dietary iron, presumably in response to the increased demand from an expanded pool of erythrocyte precursors. However, in most of such disorders transfusion therapy is needed; in that case the contribution of increased enteral absorption to iron overload is relatively small. Iron absorption is increased in the hemolytic state, although a significant iron overload (in the absence of over-transfusions) is commonly evident only when heterozygosity for HHC or ineffective erythropoiesis coexists. A puzzling finding is the apparent relationship between splenectomy and increased absorption and accumulation of iron in hemolytic patients. Increased dietary iron from traditional beer brewed in steel drums has been regarded as the sole cause of iron overload in African populations, but recent data suggest that a genetically determined defect in the regulation of iron absorption exists in these patients and that this defect is necessary for the development of iron overload. According to the model presented in that study, heterozygotes for the iron-loading locus develop iron overload only in the face of high dietary iron, but homozygotes may become iron-loaded without increased dietary iron.

Similarly, increased iron absorption has been regarded as a cause of liver iron overload in chronic hepatic disorders (see below). However, it is possible that the coexistence with heterozygous HHC may further increase iron absorption in these patients.

Iron absorption is increased in the hypotransferrinemic mouse, probably as the result of the iron deficient erythropoiesis. Although not proved, this probably occurs also in congenital hypotransferrinemia in humans. In systemic hemosiderosis associated with aceruloplasminemia, the cause of iron overload does not primarily depend on defective regulation of iron absorption, but it is likely that the mechanisms that normally inhibit iron absorption as iron storage increases do not operate in this rare disease, due to the low serum iron levels secondary to the impaired iron release from cells to plasma. However, functional studies on iron metabolism in this human hereditary disorder have not yet been reported.

Cases of iron overload induced by prolonged oral administration of iron-containing compounds are exceptionally rare and are probably the results of an interaction with other genetically or acquired disorders able to increase intestinal iron absorption.

Parenteral route

Transfused blood is disposed through the macrophages of the reticuloendothelial (RE) system, which breaks down the hemoglobin of ingested erythrocytes. Thus, large amounts of iron accumulate as clumps of hemosiderin within the RE cells and excess iron, by some means, makes its way into the extracellular fluid and in the plasma until the capacity of apotransferrin to take up iron becomes saturated. At this point excess iron is delivered to the hepatocytes and other parenchymas leading to the development of organ damage.

Iron overload from parenteral administration of iron compounds is rare and is the result of inaccurate determination of body iron stores.

Placental route

Throughout pregnancy, iron is taken up from maternal plasma by trophoblasts and transferred into the fetoplacental circulation. This process is thought to accelerate during the last trimester. In the fetus, the hepatocyte is the main site of storage iron. Thus, based on the tests used in adults, the term infant is markedly iron overloaded and iron stores may even increase within the first post-natal weeks. It is then difficult to dissect whether systemic or liver diseases that develop during fetal or neonatal life are merely superimposed on an otherwise physiological iron overload status or they are the result of excess transplacental delivery of iron. Perinatal or neonatal hemochromatosis is a syndrome of severe idiopathic liver disease of fetal onset associated with marked hepatic and extra hepatic siderosis that is not a variant of HHC. It has been argued that this disease is due to abnormal iron handling in the throphoblast and fetal liver, but no specific defect has yet been identified.

Sites of iron accumulation

Parenchymal vs. RE iron overload

The different distribution of iron within parenchymal or RE storage areas indicates different pathogenetic mechanisms of iron accumulation and has relevant implications for organ damage and prognosis of the patients (Table 1). In fact, organ damage is related to the amount of iron present in the parenchymal cells, whereas iron within RE cells appears to be relatively innocuous. Liver parenchymal iron overload is usually the result of excessive iron absorption by the enteral route, such as in HHC and anemias with ineffective erythropoiesis (iron loading anemias), but may also reflect enhanced internal redistribution of transfused erythrocyte iron recycled from the RE cells, as observed in the more advanced stage of transfusional iron overload. In hypotransferrinemic mice as well as in congenital hypotransferrinemia, tissue iron distribution is almost identical to that seen in HHC. There is no accumulation of iron in macrophages either in bone marrow or spleen. This indicates that transferrin is not required for the recycling of iron by macrophages and that any conditions, genetic or environmental, that lowers plasma apotransferrin levels (e.g. advanced liver cirrhosis) will result in increased parenchymal iron uptake and eventual tissue damage.

RE iron overload is the result of enhanced phagocytosis of red cells (chronic hemolysis and transfusions) and uptake of iron-containing compound.
In the liver, iron may accumulate in Kupffer cells because of phagocytosis of dead iron-laden hepatocytes (sideronecrosis) or uptake of ferritin and hemosiderin from damaged hepatocytes. In inflammatory disorders, iron accumulates in macrophages because of the RE iron block; this disease state is characterized by a low to normal transferrin saturation and a progressive increase in serum ferritin and marrow hemosiderin with time. In African and African-American iron overload, iron deposition is similar to that seen in transfusional iron overload: iron is deposited equally in the spleen, bone marrow and both liver parenchymal cells and macrophages. A full explanation of the accumulation of iron in Kupffer cells and macrophages in this form of iron overload is still lacking. In both aceruloplasminemia, and ascorbic acid deficiency there is impaired delivery of iron from iron-storing cells. Decreased iron transfer to plasma or entry of iron into ferritin, but not into transferrin. Conversely, in aceruloplasminemia, in which iron overload depends on a defect on cellular export of iron, brain is a main site of iron accumulation together with liver and pancreas. Accordingly, the clinical features are characterized mainly by progressive dementia, extrapyramidal symptoms, cerebral ataxia and diabetes mellitus.

Iron accumulation can be confined to a specific organ. In several chronic hepatic disorders (alcoholic liver disease, chronic viral hepatitis, porto-caval shunts, liver cirrhosis, porphyria cutanea tarda), mild to severe iron overload is frequent in the liver, both in parenchyma and RE cells, but iron deposits are minimal in extra hepatic sites. In Gaucher’s disease, there is a marked iron accumulation in Gaucher’s cells. In late stages of Alzheimer’s disease and other degenerative neurological disorders, excessive amounts of iron have been found in some areas of the brain, where iron appears to be a component of neuronal cell death. In some rare conditions, such as idiopathic pulmonary hemosiderosis, renal hemosiderosis and superficial siderosis of the central nervous system, iron accumulation is the result of sequestration and degradation of hemoglobin from damaged erythrocytes by macrophages because of inflammatory, hemolytic or hemorrhagic processes strictly confined to a specific organ.

Causes of iron overload
From a general point of view, iron overload can be classified as primary or secondary depending whether it results from a primary defect in the regulation of iron balance or is secondary to other genetic or acquired disorders. In some cases the specific cause of iron overload has already been defined, whereas in others the mechanism leading to iron overload is not yet elucidated and these iron overload disorders remain idiopathic.

Primary iron overload
The best-known example of primary iron overload is HHC, in which iron is absorbed in excess because of increased iron transfer from the enteral cells to the blood. Recent advances in molecular genetics, clinical aspects and diagnosis of HHC have been previously reviewed in this journal. A new gene, now called HFE, has been isolated and two missense mutations, C282Y and H63D, have been identified. Whereas C282Y mutation in the homozygous state seems undoubtedly sufficient to cause HHC, some confu-
sion exists regarding the H63D variant. However, several data prove the relationship of the H63D mutation to the disease and that compound heterozygotes of this mutation with the C282Y variant are at special risk for the development of HHC, but with a low penetrance and milder phenotype expression.

A variable proportion of patients with a HHC phenotype did not show any mutation in the HFE gene in different populations. It is hypothesized that some of these patients may represent forms of non-HFE related hemochromatosis in which a primary defect, different from that observed in HHC, exists. Accordingly, recent data showed that juvenile hemochromatosis is not linked to HFE gene, and that African iron overload is caused by an interaction between high dietary iron content and a common iron-loading gene not linked to the HLA locus. These results suggest that defects of other still undefined genes may be responsible for primary derangement of iron metabolism in humans.

Secondary iron overload

This group includes iron overload either due to or associated with ineffective erythropoiesis, chronic liver diseases, parenteral administration or ingestion of excessive amounts of iron. In some of these disorders it is possible, as previously mentioned, that interactions with causes of primary iron overload exist.

Thalassemia major and sideroblastic anemia are the two best studied examples of iron overload secondary to blood transfusions and ineffective erythropoiesis. Because abnormalities in hemoglobin can decrease erythrocyte life span, the pool of erythrocyte precursors is markedly expanded in certain hemoglobinopathies, leading to increased enteral absorption of dietary iron.

Aggressive transfusion therapy suppresses endogenous erythropoiesis and corrects the severe anemia, but leads to its own complications, the worst of which is iron overload. Sideroblastic anemias are a heterogeneous group of inherited and acquired hematopoietic disorders characterized by the association of anemia with the presence of non-heme non-ferritin iron deposits within the mitochondria of erythroid precursors in the bone marrow (ringed sideroblasts). It has been generally assumed that mitochondrial iron deposits are secondary to the failure of heme synthesis that would lead to ineffective erythropoiesis and increased iron absorption.

The main causes of iron overload in chronic hepatic diseases are shown in Table 2. In these cases liver iron overload is frequent, often mild or moderate, and iron deposits are usually found in both Kupffer cells and hepatocytes. In these circumstances, even relatively low amounts of iron may amplify and propagate the initial toxic effect of alcohol and viruses, with rapid acceleration of the fibrotic response in the liver. In porphyria cutanea tarda, liver iron overload is the result of an interaction between several factors: heavy alcohol intake, chronic viral hepatitis and heterozygosity for the HFE gene mutations. Iron overload in end-stage liver cirrhosis is a rather frequent condition characterized by a continuum from slight and spotty iron deposition to diffuse and marked siderosis. This post cirrhosis siderosis seems to be acquired rather than genetically determined. However, data on HFE gene mutations in these patients are lacking.

A new syndrome of liver iron overload has been recently described as generally modest, associated with increased ferritin, normal transferrin saturation and presence of metabolic disorders. The mechanism leading to parenchymal iron overload in these patients is still undefined.

### Diagnosis of iron overload

Iron overload is suspected when biochemical iron indices are increased and confirmed by the demonstration of increased iron deposits either by liver biopsy, magnetic resonance imaging (MRI) and spectroscopy or subsequent quantum interference device (SQUID), or by retrospective evaluation of total iron removed by phlebotomy. The differential diagnosis among the different causes of iron overload could be difficult and should take into account clinical, biochemical and histological data. The recent identification of the HFE gene mutations added a simple and strong tool in the differential diagnostic strategy of iron overload conditions. Figure 1 illustrates a practical approach to the diagnosis of iron overload disorders.

### Biochemical iron indices

Three iron markers can be considered: serum iron, transferrin saturation and serum ferritin. Serum iron...
has no value *per se* in the diagnosis of iron overload, but it remains an obligatory index because it is necessary for measuring transferrin saturation. Approaching the diagnosis of iron overload disorders, one should consider that some iron indices might be elevated in non-iron overloaded situations. Moreover, in some iron-overload disorders transferrin saturation and serum ferritin may behave differently in relationship to the different mechanism leading to iron overload. Figure 2 schematically shows the different mechanisms leading to high transferrin saturation and/or serum ferritin values in iron overload disorders.

**Transferrin saturation**

It corresponds to the ratio of serum iron and total-iron binding capacity. However, in most laboratories, total-iron binding capacity is not directly measured, but is deduced from serum transferrin concentration, after correction for a constant value (mg of transferrin multiplied by 1.24 or 1.25).27,50 Thus, there is a need for homogeneity among different laboratories.

Transferrin saturation is influenced by the same variations of serum iron (within day and day to day variability and inflammation), which can limit its clinical usefulness.25 In *non-iron overloaded* conditions transferrin saturation might be high in presence of liver dysfunction, due to increased serum iron (through hepatocellular necrosis) and decreased transferrin synthesis (through liver failure).17,19,23 However, with time this condition may be a cause of hemosiderosis secondary to liver cirrhosis, due to the increase of non-transferrin bound iron in the plasma (see first section of the review).19,21 In *iron overload* situations transferrin saturation is usually elevated (>50%) before serum ferritin increases. It can be lowered by ascorbic acid deficiency (iron-excess related),18,20 is low in hereditary aceruloplasminemia,23 normal in the recently described iron overload syndrome associated with dysmetabolic disorders48 and in Gaucher’s disease (see below).34

**Serum ferritin**

It is increased in a number of *non-iron overloaded* situations, including infections, acute and chronic inflammatory disorders, hepatocellular necrosis, alcohol abuse (through hepatocellular necrosis and increased ferritin synthesis), and deregulation of L-ferritin synthesis (hereditary hyperferritinemia-cataract syndrome).1,2,51 In *iron overload* situations serum ferritin is usually higher than the upper normal limits corrected for sex and age (Table 3) and is most often associated with high transferrin saturation,7,24 excluding those situations previously mentioned.18,20,23,34,48 In dysmetabolic associated liver iron overload, serum ferritin levels overestimate body iron stores.48 Serum ferritin concentrations are usually related to the amount of body iron stores, but a variety of condi-
tions that may frequently associate with iron overload, may reduce (ascorbate deficiency), or increase serum ferritin levels (hepatocellular necrosis either iron-induced or related to coexistent alcohol abuse or viral infections), independently of changes of body iron burden. In hemochromatosis, ferritin concentrations above 1000 µg/L suggests liver damage (fibrosis or cirrhosis). The same level is associated with an increasing risk of developing iron-induced complications in thalassemia major.

### Assessment of iron overload

#### Liver biopsy

It is still essential in the diagnostic pathway of iron overload disorders. Liver biopsy ascertains iron overload, defines its distribution within the hepatic lobules, provides a semi-quantitative evaluation of iron excess by different grading systems, enables the quantitation of iron by measurement of liver iron concentration (LIC), and informs on the degree of tissue iron-dependent damage and associated lesions (i.e., alcoholism, chronic viral hepatitis, steatosis). The distribution of iron may vary in different iron overload disorders and is related to the underlying mechanisms responsible for iron accumulation (see also paragraph Site of iron accumulation). Sometimes, liver iron distribution can be more useful that LIC in differential diagnosis of iron overload disorders. Non-homogeneous iron deposition within the liver without intrabiliary iron deposits is the typical pattern of iron overload associated with alcoholic and post-hepatitis cirrhosis. In HHC as well as in iron overload secondary to ineffective erythropoiesis, iron is deposited in the liver in a lobular distribution with a decreasing periportal-to-pericentral gradient.
whereas in transfusional and African iron overload, iron deposition is panlobular and typically involves Kupffer cells and portal macrophages. The upper normal limit of LIC ranges between 30 to 39 µMol/g in men and 18 to 28 µMol/g in women, in different studies. In classical forms of iron overload either primary or secondary, LIC is generally higher that 90 µMol/g in men and 60 µMol/g in women. The hepatic iron index (HII), defined by Bassett et al., as the LIC to age (years) ratio, has been widely accepted as a tool for differentiating HHC patients from either heterozygotes and patients with alcoholic siderosis. Currently, many authors assume that in the absence of a cause of secondary iron overload, a HII greater than 1.9 or 2 is suggestive of HHC. However, this assumption is questionable when advanced cirrhosis is present, due to the wide intrahepatic variability of iron concentrations found in this condition.

**Future directions**

The discovery of the HFE gene mutations provides the opportunity for a more precise classification of those heterozygous and homozygous for HHC. Moreover, HFE molecular testing will allow the clarification of the role of both mutations in some iron overload conditions of uncertain origin. It has been previously suggested that the heterozygous state for HHC may favor the development of significant iron overload in several diseases such as chronic hemolytic disorders, porphyria cutanea tarda, hemoglobinopathies, or chronic liver diseases and that HHC heterozygotes may be at an increased risk of malignancies. The availability of the molecular analysis of HFE gene is an important tool to test some of these associations.

The causes of iron overload remain to be clarified in several disorders (e.g. African-American iron overload, dysmetabolic associated iron overload, perinatal and overload disorders. In normal subjects IR is below 1.5 g, whether in major iron overload situations is generally higher than 5 g.

Many studies show that MRI can reflect the presence of tissue iron in vivo, but this method has not been validated as one that provides measurements of tissue iron that are quantitatively equivalent to those determined at liver biopsy. Conversely, SQUID is a rapid, safe, reliable and quantitative measurement of liver iron overload, but the instrumentation for this technique is scarcely available. However, none of these techniques provide information regarding iron distribution and histological findings.

**Molecular analysis of HFE gene**

In the presence of increased biochemical iron indices, once that major causes of secondary iron overload has been excluded, searching for the two HFE gene mutations should be the next and simplest diagnostic step. Homozygosity for the C282Y mutation is generally associated with expressed HHC with few exceptions. Table 4 shows the relationship between HFE genotypes and phenotypes, as deduced from data presently available (refs #5, 39, 41, Proceedings of the International Symposium on Iron in Biology and Medicine, S. Malo, France, June 16-20, 1997, and ref. #54).

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The causes of iron overload remain to be clarified in several disorders (e.g. African-American iron overload, dysmetabolic associated iron overload, perinatal and
juvenile hemochromatosis, post-cirrhosis hemosiderosis) and is hypothesized that both acquired and genetic factors are involved. Some studies suggest the existence of forms of genetically determined, non HFE-related hemochromatosis that await the identification of their own molecular defects. Genes other than HFE are likely to be involved in the regulation of iron absorption, as recently shown by the cloning of iron transporters in mammalians.

The recent description of a new condition associated with liver iron overload that seem to be related to metabolic disorders, open new perspectives on the possible link between iron excess and atherosclerotic and cardiovascular disorders and raises questions about the mechanisms of parenchymal iron loading in the presence of normal transferrin saturation. Further studies are needed to clarify the mechanisms leading to iron overload in patients with chronic viral hepatitis and end-stage liver disease and to understand the role of HFE gene in these disorders. In addition, based on the observation that increased liver iron concentrations may favor the progression to liver fibrosis in chronic viral hepatitis and negatively influence the response to interferon therapy, careful studies and follow-up are needed to clarify whether early identification and treatment of iron overload may have a beneficial effect in the natural history of these common disorders.

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