E-Book

Hereditary and Acquired Iron Overload

Editors: Pierre Brissot and Syed A. Abutalib

CHAPTER 3

HFE-HEMOCHROMATOSIS: DIAGNOSIS AND TREATMENT

AUTHORS: Brissot Pierre\textsuperscript{1,2}, Cavey Thibault, \textsuperscript{2,3}Ropert Martine\textsuperscript{2,3}, Guggenbuhl Pascal, \textsuperscript{2,4}Loréal Olivier\textsuperscript{2}

AFFILIATIONS:

(1) Hepatology. Faculty of medicine. University of Rennes 1. Rennes (France)
(2) Inserm-UMR 991. Rennes (France)
(3) Department of specialized biochemistry. University hospital of Rennes. Rennes (France)
(4) Department of rheumatology. University hospital of Rennes. Rennes (France)

CORRESPONDENCE:

1. **Introduction**

The term hemochromatosis, which corresponds to systemic iron overload of genetic origin, encompasses a number of genetic entities whose clinical expression, diagnostic and therapeutic management can share many aspects but also markedly differ. From the genetic viewpoint, the major boundary concerns the involvement or not of the HFE gene, thus leading to distinguish between HFE-hereditary hemochromatosis (HFE-HH) and non HFE HH. After recalling some important pathophysiological data, we will consider here HFE-HH (also named type1 hemochromatosis) in its diagnostic and therapeutic aspects. HFE-HH affects only Caucasian populations and represents the most frequent form of hemochromatosis, affecting at least 1 person per thousand (with an approximate mean of 3 per thousand).

2. **Pathophysiological aspects(1)**

The HFE gene, identified by Feder et al. in 1996, is located on the short arm of chromosome 6. Its major mutation C282Y (novel denomination p.Cys282Tyr), when present in the homozygous state (C282Y/C282Y), may lead, via a molecular cascade that is not yet fully understood, to the decreased production by the hepatocytes of the iron hormone hepcidin(2-4) and subsequently to decreased plasma hepcidin levels. Knowing that hepcidin down-regulates the activity of ferroportin, the only known cellular iron exporter, the resulting hypohepcidinemia enhances the iron release from the enterocytes during intestinal absorption and from the spleen macrophages during the erythrophagocytotic process. As a consequence, plasma iron and plasma transferrin saturation (TfSat) increase, and non-transferrin bound iron (NTBI) appears in the plasma. NTBI, in contrast with transferrin-iron that is mainly directed to the bone marrow space, targets the parenchymal cells, and in first line those of the liver (hepatocytes) ([Figure-1](5)). Moreover, one of the NTBI forms, defined
by its property to generate reactive oxygen species (ROS) and called LPI (labile plasma iron)(6) or RPI (reactive plasma iron) represents the potentially toxic form of circulating and cellular iron, and is considered as the main culprit for cellular, and therefore tissue and organ damage, in hemochromatosis. Importantly, C282Y homozygosity is necessary but not sufficient to produce the disease. This partial penetrance raises the key issue of identifying the genetic and/or acquired co-factors which modulate genotypic expression. Moreover, C282Y is not the sole HFE mutation: H63D heterozygosity (p.His63ASp) is frequently observed (15-20% of the populations) but should be considered as a simple polymorphism. Rare HFE mutations, especially as part of compound heterozygosity (C282Y/rare HFE mutation), may lead to clinical HH(7). The sequence C282Y homozygosity - hypohepcidinemia - hypersideremia is the basic pathophysiological triad underlying HH.

FIGURE 1: Liver biopsy in type1 hemochromatosis (Perls staining). Blue iron deposits within the hepatocytes (arrow). By courtesy of Dr B. Turlin (Pathology department, University hospital of Rennes).
3. Diagnostic aspects (8, 9)

3.1. Clinical presenting symptoms

3.1.1. First notion. Clinical expression can be totally absent. Indeed, C282Y homozygosity produces symptoms usually only during late adulthood (30 and 40 years old in males and women, respectively). HFE-HH is most often a clinically delayed genetic disease. During this asymptomatic phase, the only possibility for diagnosing HH predisposition in a given Caucasian individual would be, ideally, to systematically check for plasma abnormalities of iron parameters (meaning TfSat and ferritin, since TfSat increase is the earliest marker of iron metabolism abnormality and hyperferritinemia reflects body iron overload—see below).

3.1.2. Second notion. When present, clinical expression is very heterogeneous. Two symptoms are particularly frequent. The most frequent one is chronic fatigue which may rather lead to search for iron deficiency. Erectile dysfunction may be part of this syndrome. The main pitfall would be then to relate these symptoms to a psychological origin. The other frequent symptom concerns the joints (10). Pains can affect any joint as acute or chronic mono- or poly- arthritis. They are frequently misdiagnosed as rheumatoid arthritis or primary osteoarthritis, and the diagnostic delay may reach 5 to 10 years after the onset of the symptomatology. The following rheumatological features should draw medical attention: pain location at the second and third metacarpophalangeal joints, responsible for a highly suggestive painful handshake (Figure 2), and the discovery of chondrocalcinosis or osteoarthritis in people younger than 50 or in joints where there is no primitive osteoarthritis such as metacarpophalangeal ones, shoulders or ankles. Other clinical features can be melanodermia, hepatomegaly with mild cytolysis (transaminases not more than 2-3 times the upper normal limits) sometimes cirrhosis and hepatocellular carcinoma, diabetes, osteoporosis (with fractures), and cardiac symptoms (rhythm disturbances, rarely cardiac failure). Diagnosing HH is difficult due to the myriad of possible presenting symptoms, either isolated or diversely associated.
3.2. Biological presenting sign

Hyperferritinemia is the key biochemical abnormality leading to suggest iron overload. Its interpretation must however be very rigorous. Indeed, the most frequent cause of hyperferritinemia is metabolic syndrome(11). Following features may be, more or less associated with metabolic syndrome: increased body mass index and waist circumference, increased blood pressure, hyperlipidemia, hyperglycemia, and hyperuricemia (with sometimes gout). In this setting, hyperferritinemia is associated with normal plasma TfSat (<45%). Hyperferritinemia can also be related to alcoholism or inflammation. It is therefore essential to rule out these possible confounding factors before considering that hyperferritinemia does reflect body iron excess. The most frequent cause of hyperferritinemia is the metabolic syndrome.

3.3. Diagnostic strategy (Figure 3)

3.3.1. Search for hepcidin deficiency phenotype through TfSat study. Once evoked from clinical and/or biological data, the subsequent diagnostic step is to search for increased plasma TfSat. Finding such an increase (TfSat >45% and often >60%, reaching up to 100%) is

***FIGURE 2**: Typical hemochromatosis rheumatism. Red ovals indicate inflammatory areas at the levels of metacarpophalangeal and proximal interphalangeal joints
critical for diagnosing HFE-HH since it represents the basic and earliest biochemical abnormality of the disease. Normal plasma TfSat excludes the diagnosis of HFE-HH. There is, however, one exception: the fortuitous simultaneous occurrence of an inflammatory syndrome which decreases (and can even normalize) transiently TfSat levels. It is therefore of good clinical practice to check CRP (C-reactive protein) whenever TfSat is measured. In the absence of inflammation, normal transferrin saturation rules out HFE-HC.

3.3.2. Demonstration of parenchymal iron overload. Visualizing tissue iron overload is an important step. It does not resort anymore to liver biopsy which has been replaced by the noninvasive MRI (magnetic resonance imaging) technique. MRI to detect hepatic iron stores can be performed by various techniques. Some of them consist of relaxometric methods determining indices such as T2* or R2*(12). In our hands, the most practical technique uses the signal intensity ratio (SIR) approach (13). Based on the comparison of the hepatic signal to the paraspinal muscle signal (which serves as a control), this technique does not require specific MRI device and produces a reliable determination of hepatic iron concentration as shown by the good correlation between the decreased T2 signal and HIC increase. Moreover, SIR allows to assess also splenic and pancreatic iron concentration. The determination of splenic iron concentration has a great interest in the diagnostic process of HFE-HH. Indeed, in this disease, there is a marked contrast between increased HIC (due to hepatocyte iron deposition) and the absence of splenic iron excess (due to the increased iron release from the spleen into the plasma). Therefore, schematically, the typical T2 MRI profile of HFE-HH is a “black liver and a white spleen” (Figure4). In HFE-HH iron-MRI shows the contrast between increased hepatic iron concentration (black liver) and absence of splenic iron overload (white spleen). However, iron-MRI is not always available and, in the absence of co-factors likely to increase serum ferritin, represents a desirable but dispensable diagnostic step.

3.3.3. The genetic nature of iron overload can then be established?
Family data, if present, are of course valuable to suggest hemochromatosis. It is also essential to exclude other iron overload situations. Transfusional iron overload is usually easily diagnosed on the context of chronic anemia requiring multiple blood transfusions such as in hemoglobinopathies or myelodysplasia. Excessive parenteral iron supplementation is another easily recognizable setting.

The overall collected data allows then to perform genetic testing with the expected result of C282Y homozygosity. Increased TfSat + C282Y/C282Y = HFE (type1)-HC. One frequently raised question concerns the diagnostic interest of searching for the H63D
mutation. The European Molecular Quality Network (EMQN) guidelines (14) are the following: in case H63D mutation has been determined: simple heterozygosity has no pathological significance. The presence of compound heterozygosity (C282Y/H63D) or H63D homozygosity (H63D/H63D) does not expose to the risk of clinically expressed iron overload (at most increased TfSat) but may represent a cofactor of iron excess in situations such as the dysmetabolic syndrome and alcoholism. C282Y/H63D or H63D/H63D profiles, taken alone, do not expose to the risk of clinically significant iron overload. It should be noticed that absence of C282Y homozygosity does not definitively close the possibility of HFE-HH since, as previously mentioned, some exceptional compound heterozygosity profiles can produce a typical type 1-HC phenotype. In addition, rare mutations in other genes inducing an hepcidin deficiency syndrome must be also considered: hemojuvelin (HJV or HFE2), hepcidin itself (HAMP), or transferrin receptor 2 (TFR2).

3.3.4. A global check-up of the bio-clinical expression of HC must be performed, assessing the following organs: i) the liver: liver function tests, ultrasound examination, if possible transient elastography for evaluating hepatic fibrosis, rarely now liver biopsy; ii) symptomatic joint and bone: x-rays, osteodensitometry; iii) endocrine system: glycemia, testosterone; iv) the heart: electrocardiogram, echocardiography, cardiac MRI. This check enables to classify the patient in one of the five classes of C282Y-C282Y expression (stage 0: neither clinical nor bio-clinical expression; stage 1: only increased TfSat; stade 2: increased TfSat + increased ferritin (corresponding to the indication for phlebotomies); stade 3: = stade 2 plus clinical signs affecting the quality of life (fatigue, impotence, arthropathy); stade 4: = stade 3 plus potentially life-threatening syndromes (liver cirrhosis, insulin-dependent diabetes, cardiomyopathy).
FIGURE 3: Schema of overall sequential management of hemochromatosis. TfSat: transferrin saturation. MRI: magnetic resonance imaging.

FIGURE 4: Iron-MRI (magnetic resonance imaging) in type 1 hemochromatosis (T2 signal intensity ratio technique). Red arrow: spinal muscle (=control zone); Yellow arrow: «black liver» (iron
4. Therapeutic aspects

This section will focus on iron overload treatment.

4.1. Venesection therapy

4.1.1. Rational

Given the high amount of iron within the red blood cells of approximately 2 grams (half the total quantity of body iron), removing erythrocytes is an ingenious way to force the body to pump up its iron reserves in order to produce new red blood cells. Moreover, hepcidin deficiency increases ferroportin activity, implying increased export of intracellular iron, and therefore an efficient recycling process.

4.1.2. Overall scheme

It is a two-step process. The first phase, called induction phase, consists of removing the established iron overload at the time of diagnosis. The second phase, which lasts theoretically lifetime, aims to prevent iron re-accumulation.

4.1.3. Practical modalities

Venesections can be performed in various places (hospital, clinic, general practitioner office, nurse office, patient home). The patient is non-fasting, comfortably sitting or lying. Thanks to a bleeding kit, blood is removed over a 15-20 minute period. The French recommendations are, for the induction phase, to withdraw, on a weekly basis, 7 ml/kg body weight of whole blood, without exceeding 550 mL, until plasma ferritin levels (checked every month until the ferritin levels reach the upper limits of normal) reach 50 µg/L and subsequently twice a month. A snack should be given at the end of the procedure and the patient must drink a liquid volume equivalent to the blood withdrawn. The schedule of maintenance therapy depends on individual patient. It is usually determined after a few months of follow-up, and consists of one venesection every, 1 to 3 months. Serum ferritin levels should be checked after every two venesections, and hemoglobin level verified prior to the every phlebotomy. One
frequently raised question concerns the interest to check TfSat. The first notion to keep in mind is that the essential objective is to maintain plasma ferritin around 50µg/L which always means that no body iron excess is present. The primary goal of venesections is to obtain and maintain ferrinemia close to 50 µg/L. As to TfSat, it is often elevated despite normal ferrinemia because it reflects the natural tendency of hemochromatosis patients to hyperabsorb dietary iron and release iron from macrophages, a tendency that can be accentuated by the venesection process itself. When moderately elevated (TfSat <75%) the pathological significance is very unlikely. However, if constantly over this value, it may correspond to the presence of NTBI, and mostly LPI (RPI), and therefore might be deleterious. It is why, in our view, it is advisable to check TfSat, for instance twice a year, in order to ensure that the patient biochemical profile is not permanently that of a very high TfSat despite normal serum ferritin levels.

4.1.4. Results

- Tolerance. It is good, but not perfect, as shown by an international questionnaire that pointed out that a significant percentage of patients experienced some side effects(15).
- Efficacy. Usually excellent, with disappearance or marked attenuation of fatigue, hyperpigmentation, cytolysis and (moderate) fibrosis. However, the joint symptoms are often refractory to venesection therapy and can even become worse. Joint symptoms are often refractory to venesection therapy. Moreover, when liver cirrhosis was present at the time of diagnosis, removing completely the iron excess does not exclude the risk of subsequent development of hepatocellular carcinoma. Cirrhotic patients need therefore to be checked, twice a year, for serum alphafetoprotein levels and mostly by ultrasound hepatic imaging. Venesection therapy is a simple, cheap, well-tolerated and efficient treatment.

4.2. Other therapeutic options

4.2.1. Erythrocytapheresis(16). Consisting of selective withdrawal of red blood cells, this procedure, although more complex and more expensive than whole blood phlebotomy, is more efficient and globally well tolerated.

4.2.2. Chelation therapy. Its exceptional indications correspond to phlebotomy contraindications (psychological reluctance) or technical infeasibility (poor venous access). Rather than using prolonged subcutaneous injections of desferrioxamine via a portable pump device that corresponds to a demanding procedure, oral chelation (deferasirox)(17) may be proposed but corresponds, in hemochromatosis, to an off-label
compound which must, therefore, be prescribed under doctor’s personal responsibility and with a written informed consent by the patient.

4.2.3. **Alimentary measures.** A general iron-deficient diet is not usually recommended. However, vitamin C medical supplementation must be avoided (mainly because ascorbic acid increases iron absorption). In contrast, tea supplementation may be beneficial since it decreases iron absorption.

4.2.4. **Hepcidin supplementation** (18). It represents the pathophysiologically relevant therapeutic approach and a recent study, in HC liver transplanted patients, has provided the clinical proof of concept for this innovative approach. However, the best modalities of hepcidin supplementation (exogenous administration versus stimulation of its endogenous synthesis) remain to be determined.

5. **Prevention**

It should be considered at two main levels.

5.1. **Family prevention**

As soon as a given individual has been diagnosed, it is essential to engage a family study. HH being a recessive disease, the siblings are the most exposed relatives and therefore must always be checked (after legal majority). However, due to the high prevalence of $C282Y$ heterozygosity (at least one in 10 Caucasian persons), the children should also be checked (after legal majority). The key parameter is the genetic test: family members devoid of the $C282Y$ mutation, or carrying only one mutation (heterozygotes), are not at risk for HC and are, therefore, free of any follow-up. Heterozygotes persons should however be informed that statistically half of their children will be heterozygotes, and that children may be homozygotes in case their other parent is also an heterozygote. As to the relatives detected as $C282Y$ homozygotes at the time of the family study, either they have already developed the disease or they are at risk of developing it and need to be followed in terms of their plasma iron parameters. It should be recalled that $C282Y$ homozygosity is not synonymous of HH since it is a necessary but not sufficient condition for developing the clinical disease. As long as criteria of disease predictability have not been identified, only a systematic bioclinical follow-up can be proposed. The family study is critical as soon as a given HC patient has been diagnosed.

5.2. **Mass screening prevention**

Theoretically, type1 HH gathers all the criteria for proposing a systematic screening of the population for HC. Indeed, it is a frequent and easily diagnosed disease that, moreover, benefits from a simple, cheap and efficient treatment, an exceptional situation in the field of
Two issues remain however to be clarified before adopting such a population screening policy: i) the methodological approach. Checking for the C282Y mutation is difficult to propose, at least as long as predictive criteria for developing the disease have not yet been determined. Indeed, it appears risky, from both individual and societal viewpoints, to label individuals as potential patients in a disease with such a partial penetrance. The biochemical approach seems preferable as there is an early and simple plasma marker of disease expression, namely increased TfSat. ii) The debated ratio of financial cost versus health benefit. In this regard, an Australian study(19), that represents the most in-depth economic analysis so far provided in this disease, concludes that adopting a policy for reducing the consequences of HH would likely lead to substantial reductions in cost. Population screening for HH should remain a major objective.

6. Expert summary. Practical and applicable points
- HFE-HH (type 1 HH) is one of the most frequent genetic diseases in Caucasian populations
- HH is mostly related to C282Y mutations of the HFE gene
- C282Y homozygosity is necessary but not sufficient for disease expression
- The diagnosis is noninvasive, based on combined clinical, biological and imaging approaches
- Phlebotomies remain the mainstay of iron overload treatment
- Cirrhotic patients must be regularly followed (even after iron overload removal) : serum α-fetoprotein (AFP) levels and ultrasound hepatic imaging twice a year
- Family prevention is critical

7. Future directions
- To improve our understanding of the precise molecular cascade linking the HFE mutation and the decrease of hepcidin synthesis
- To extend the access to iron-MRI which is a key noninvasive tool not only to ascertain and quantify iron overload but also to guide its pathophysiology (liver versus spleen iron balance)
- To set up the modalities for hepcidin supplementation that represents the “pathophysiological » therapeutic” approach of HH
- To pursue, with the help of patient associations, the information on the disease towards physicians (GP and specialists), health authorities, the public and the medias
- To set up population screenings for this frequent, easily diagnosed and efficiently treated genetic disease
References