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## **Optimising the diagnosis and the treatment of iron overload diseases**

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### **Abstract**

A number of human disorders are related to chronic iron overload, either of genetic or acquired origin. The multi-organ damage produced by iron excess leads, in adults and in children, to severe clinical consequences, affecting both quality of life and life expectancy. The diagnosis is increasingly based on a non-invasive strategy, resorting to clinical, biological and imaging data. The treatment rests on either venesection or chelation therapy, depending on the etiology. Major advances in the fields of molecular biology, pharmacology, and biotechnology pave the road for key improvements in the diagnostic and therapeutic management of the patients.

Iron overload diseases encompass a large spectrum of genetic and acquired disorders. The symptoms induced by chronic iron excess are close, whatever the etiology, and may cause significant morbidity and mortality representing a worldwide health problem. The diagnosis of iron overload is now based on a non invasive approach, resting on clinical, biological and imaging data. The treatment, which depends on the mechanisms underlying the development of iron overload, is mainly based on venesection or chelation therapy. However, in light of major advances in the pathophysiological understanding of these diseases(1), a number of promising innovative therapeutic approaches are emerging.

## 1. SPECTRUM OF IRON OVERLOAD DISEASES (Fig.1)

### 1.1. CAUSES

#### 1.1.1. GENETIC IRON OVERLOAD DISORDERS

They correspond to various forms of hemochromatosis (HC)(2, 3).

1.1.1.1. HFE-related hemochromatosis. Also called Type 1-HC, it is by far the most frequent form of genetic iron overload disease. The usual underlying genotype is homozygosity for the C282Y mutation(4) (new HFE terminology *p.Cys282Tyr*). Type 1 HC is exclusively present in the caucasian (white) population, with a prevalence of homozygosity close to 3 per thousand. C282Y homozygosity should be considered as a mere predisposition for the disease since phenotypic expression is quite variable (due to incomplete penetrance of the gene). It has thus been reported that approximately only 1% of homozygote women and less than 30% of homozygote men may develop a fully expressed form of the disease(5), compromising both quality of life and life expectancy. Type 1 HC is an adult disease, most often expressed after 30 and 40 years old in men and women, respectively. It should also be noted that some data have pointed out the possible physical advantage provided by HFE mutations (6, 7).

1.1.1.2. Non HFE-related HC. They are rare diseases, but present in both caucasian and non-caucasian populations(8, 9). i) Type2 HC corresponds to a severe form of iron overload occurring in younger individuals (less than 30 years old), and therefore called juvenile HC. It is due to mutations of the hemojuvelin (*HFE2* or *HJV*) gene or of the *HAMP* (hepcidin) gene (corresponding to types 2A and 2B HC, respectively). ; ii) Type3 HC is due to mutations of the transferrin receptor 2 (*TFR2*) gene. Usually expressed in adults, it may also cause juvenile HC ; iii) Type4 HC. Due to mutations of the ferroportin (*SLC40A1*) gene, it is the only form of HC with a dominant mode of transmission. In its usual form (called type A), the mutations alter the export function of ferroportin. In the type B form, the mutations alter the hepcidin receptor function of ferroportin, leading to a refractoriness state toward hepcidin ; iv) Hereditary aceruloplasminemia (HA) (see below) may also be classified among non-HFE related HC.

1.1.1.3. Compared prevalences of HFE and non-HFE HC. Types 1, 2 and 3 HC are very rare as compared to HFE-HC. It has been reported that the predictive frequency of HFE pathogenic genotypes was approximately 1/1000, versus 1/5000 000 for type 2A HC, 1/6000 000 for type 3 HC, and 1/180 000 000 for type 2B HC. Unexpectedly, the frequency for type 4 HC was close to that of type1 HC (1/1300), partly due to the dominant nature of the disease(9).

## 1.1.2. ACQUIRED IRON OVERLOAD DISORDERS

1.1.2.1. Iron supplementation. Chronic iron *deficiency* is a frequent worldwide condition(10). It may be due to iron dietary deficiency, especially in the frame of malnutrition, and requires usually oral iron supplementation. This

type of oral supplementation is not prone to induce iron overload, given the physiological limitations of duodenal iron absorption. In contrast, in parenteral iron administration, such as required in patients who undergo hemodialysis for chronic renal failure, iron excess can develop if iron therapy overcomes the needs(11).

1.1.2.2. Transfusions. Repeated transfusions are a major cause of iron overload(12).

Three main types of hematological diseases necessitate multiple transfusions : transfusion-dependent thalassemia, myelodysplastic syndromes, and aplastic anemia related to the therapeutic regimen applied in bone marrow stem cell transplantation.

1.1.2.3. Dyserythropoiesis. Whatever its hematological context, dyserythropoiesis can lead to potentially damaging body iron excess. This has been especially demonstrated in non-transfusion dependent thalassemia (thalassemia intermedia)(13).

## 1.2. PATHO-PHYSIOLOGY

### 1.2.1. IRON OVERLOAD MECHANISMS

#### 1.2.1.1. Increased cellular iron ingress :

- Hepcidin deficiency is the prevailing mechanism in types 1, 2 and 3 HC as well as in dyserythropoiesis. The common mechanistic denominator is the decreased hepatic production of hepcidin which is the master regulator of systemic iron(14-16). This decrease is due, in HC, to the involved mutations and, in dyserythropoiesis, to the action of the bone marrow hormone erythroferrone (ERFE)(17, 18). The subsequent decreased plasma hepcidin concentration induces both an increased duodenal iron absorption, and an increased release by the spleen of the iron coming from the normal degradation of senescent erythrocytes

(erythrophagocytosis). The consequence is a chronic elevation of plasma iron concentration. Part of this iron is, as physiologically expected, taken up by its plasma carrier protein, transferrin, forming transferrin-iron. Transferrin-iron is essentially delivered to the bone marrow in order to contribute to the production of new red blood cells. But, due to the high levels of plasma iron, the quantity of circulating transferrin is insufficient to bind all of the metal, so that iron becomes partially present under another form, called non-transferrin bound iron (NTBI)(19, 20). NTBI, which is likely bound to low molecular weight ligands such as citrate and acetate but also to albumin, has the very special kinetic property to be very avidly taken up, not by the bone marrow, but by various parenchymal cells, especially in the liver (hepatocytes), pancreas, and heart. Therefore, NTBI is considered as the main responsible for iron excess development in many organs.

- Hepcidin refractoriness (type4B-HC). Although plasma hepcidin concentration is not decreased, the down-stream cellular consequences are similar to those occurring in quantitative hepcidin deficiency.
- Iatrogenic excessive body iron intake : It corresponds to excessive parenteral administration of iron, either as iron *per se* (intravenous infusions) or through multiple transfusions, the erythrocytes being very iron rich (they represent half of the total body iron content).The fate of parenteral iron is to be stored within the reticuloendothelial system (macrophages), namely essentially in the spleen. This storage process is very fast for infused iron and postponed to the end of erythrocyte life for transfusional iron. Iron is subsequently released into the blood stream, leading to increased transferrin saturation and to NTBI production.

#### 1.2.1.2. Decreased cellular iron egress : Type4A-HC (also called ferroportin disease)

involves mutations which impair the iron export function of ferroportin. Ferroportin is the only known cellular iron exporter, and loss of this function leads to intracellular iron retention. In this situation, cellular iron overload affects mainly the spleen (due the high ferroportin activity at the macrophage level) and is accompanied by low plasma iron and transferrin saturation levels (since cellular iron delivery into the plasma is impaired). Another genetic disease, in which iron overload results from an impairment in cellular iron egress, is HA(21). The underlying mechanism, although incompletely understood, involves the absence of ferroxidase activity which in turn may lead to ferroportin dysfunction(22).

### 1.2.2. IRON OVERLOAD TOXICITY

#### 1.2.2.1. Role of reactive oxygen species (ROS)

Iron is known for its involvement in the Fenton reaction leading to ROS production(23). The iron species involved in this toxic reaction is not transferrin-iron, but a special NTBI component, named labile plasma iron (LPI)(24-27). This iron species is able to target cellular membranes and nuclei, and is considered the main culprit for iron-related cellular and organ damage.

#### 1.2.2.2. Special considerations

- Differential chronology of NTBI versus LPI involvement according to the mechanism of iron overload. In the setting of hepcidin deficiency (types 1, 2, 3 and 4B-HC, as well as dyserythropoiesis) or of parenteral iron injection, the appearance of plasma NTBI and LPI is an early process whereas, in transfusional iron overload, iron is first sequestered within the macrophages before being gradually released into the plasma,

generating NTBI and LPI. It means that, in transfusional iron overload, iron deposition concerns the spleen macrophages and other reticuloendothelial cells (Kupffer cells for the liver) first, parenchymal cells (especially hepatocytes) being subsequently involved.

- Iron toxicity depends on the cellular type affected by iron deposition. Thus, iron is more damaging to parenchymal cells (typically the hepatocytes), than to reticuloendothelial cells (typically the macrophages).
- The liver is a critical cross road in the process of body iron toxicity :i) as a major iron storage organ, it is capable, for a long period, to protect many other organs from becoming iron overloaded ; ii) however, when this role of reservoir is overwhelmed by the duration and intensity of iron overload, the liver may loose its « bulwark » property, opening the road for extra-hepatic iron toxicity ; iii) in chronic liver failure -whatever its etiology- the decreased production by the hepatocytes of transferrin and hepcidin(28) may favor body iron overload and toxicity. Indeed, both mechanisms contribute to increase plasma transferrin saturation (through decreased plasma transferrin concentration and increased iron concentration, respectively), leading to the formation of plasma NTBI and LPI ; iv) the liver is a preferential target of iron toxicity. Being a storage organ is in fact a double-edged sword with the risk of progressive iron-related hepatocyte damage, leading to fibrosis, cirrhosis, and even hepatocellular carcinoma.
- The role of cofactors acting as modulators of iron overload and toxicity is increasingly acknowledged. These factors can be environmental (acquired), such as menstruations or pregnancies which can contribute



to decrease iron overload, or chronic alcoholism which may aggravate hepatic iron toxicity(29). The cofactors can also be genetic. In HFE-HC, the presence of a mutation in the hepcidin promoter is associated with massive iron overload(30). Moreover, in this disease, GNAP(31), TMPRSS6(32), and PCSK7(33) polymorphisms have been reported to aggravate iron overload, to decrease iron overload, and to accentuate hepatic fibrosis , respectively.

## 2. DIAGNOSTIC ASPECTS (Fig.2)

### 2.1. GLOBAL DIAGNOSTIC STRATEGY

It is a multi-step process.

#### 2.1.1. CLINICAL APPROACH

It is the prerequisite of any diagnostic search, and should explore systematically general health and the various organs, since chronic iron overload corresponds to a systemic disease. Thus, the main syndromes, more or less associated, are : chronic fatigue, joint pains, bone demineralization, melanoderma (increased skin pigmentation), liver signs (hepatomegaly, moderate transaminase increase), diabetes, or cardiac symptoms (rhythm disturbances, cardiac failure).

#### 2.1.2. BIOCHEMICAL APPROACH

2.1.2.1. Plasma ferritin concentration. Usually defined by plasma ferritin values over 300 µg/L in men and 200 µg/L in women, hyperferritinemia is the major first-line biochemical parameter to explore total body iron stores. Whereas normal or low ferritinemia rules out iron overload, hyperferritinemia is highly suggestive of, but not necessarily synonymous with, iron overload. Indeed, it is essential to interpret rigorously hyperferritinemia since it can be related to a number of causes without significant body iron excess(34). Among those situations : i) inflammation since ferritin is an acute-phase reactant protein.

The macrophagic activation syndrome(35) is very close to this mechanism. Occurring in some infectious, hematological or auto-immune diseases it can be expressed by dramatic levels of hyperferritinemia ; ii) cytolysis, especially during acute or chronic hepatitis ; iii) alcoholism ; iv) metabolic hyperferritinemia which is likely to represent the most frequent cause of hyperferritinemia(36). Usually associated to plasma levels below 1000 µg/L, metabolic hyperferritinemia is diagnosed on a number of convergent arguments : a) normal transferrin saturation ( $\leq 45\%$ ) ; b) diversely associated metabolic symptoms, such as overweight, increased arterial blood pressure, non-insulin dependent diabetes, hypercholesterolemia, hyperuricemia, and hepatic steatosis (as shown by ultrasound examination) ; c) absent or mild liver iron overload. V) The correlation of high plasma ferritin levels with organ iron burden depends on the cellular distribution of excessive iron deposits. Indeed, it has been reported, especially when comparing transfusion-dependent and non-transfusion dependent thalassemias, that similar levels of hyperferritinemia correspond to lower hepatic iron concentrations in transfusion-dependent thalassemia. (37) This may be due to the higher propensity of macrophages to produce ferritin.

#### 2.1.2.2. Plasma iron and transferrin saturation

- Plasma iron. Its interpretation should consider some drawbacks, such as its nyctemeral cycle (with much lower values in the afternoon than in the morning), and numerous confounding factors which can either increase plasma iron(hemolysis, cytolysis), or decrease it (inflammation).
- Plasma transferrin saturation (TS). It corresponds to the ratio of iron on transferrin, and is normally less than 45%. This parameter is still too often ignored, whereas its determination (which should, at best, be

based on two successive controls) provides, in iron overloaded situations, valuable diagnostic and prognostic indications. From the diagnostic viewpoint, iron overload with elevated TS orientates primarily towards hepcidin deficiency or iatrogenic excessive body iron intake. When TS is normal or low, the iron overload etiology may be type A ferroportin disease or hereditary aceruloplasminemia. From the prognostic viewpoint, TS levels  $\geq 75\%$  are highly suggestive, in HC(25) as well as in thalassemia major(24), of plasma LPI which represents the potentially circulating toxic form of plasma iron.

2.1.2.3. As to the novel biochemical plasma iron-related parameters, such as NTBI, LPI(38) or hepcidin(39), they presently remain mostly clinical research tools.

#### 2.1.3. IMAGING APPROACH

Magnetic resonance imaging (MRI) is the reference method for direct evaluation of body iron excess(40). Various techniques have been validated, consisting of relaxometry ( $T2^*$  (41)/  $R2$  evaluation(42)) or signal-intensity ratio (SIR)(43) techniques. Although SIR cannot assess cardiac iron, it is particularly simple, quick, does not require special MRI equipment, and resorts to a software that is freely available on the web. Furthermore, SIR can, not only prove and quantify hepatic iron overload (the rate of hyposignal being positively correlated to the amount of iron excess), but also evaluate splenic iron, thus giving valuable pathophysiological informations. Indeed, the balance between liver and spleen iron overload provides a major diagnostic clue : schematically a « black » (i.e heavily iron overloaded) liver together with a « white »

spleen (no iron overload) is the typical profile observed in hepcidin cellular deprivation (related to hepcidin deficiency or refractoriness), whereas a black liver together with a black spleen is highly suggestive of transfusional iron overload. Moreover, a black spleen with a grey liver may suggest ferroportin disease.

#### 2.1.4. GENETIC APPROACH

It should be guided by the first three, above mentioned, steps. Schematically, after having excluded acquired iron overload (no parenteral iron supplementation, transfusions, or dyserythropoiesis), a careful interpretation of ferritin and transferrin saturation levels, together with the comparison of liver versus spleen MRI profiles, enables to engage relevant genetic studies(44). It must be mentioned that those specific genetic studies should be, at best, selected by clinical reference centers and require, for their determination, highly expert laboratories whose number remains very limited, stressing the importance of building appropriate international networks.

##### 2.1.4.1. Phenotype of hepcidin deprivation-related iron overload

- In a given caucasian individual, searching for the C282Y mutation is to do first. Only C282Y homozygosity is diagnostically relevant. The frequent C282Y/H63D (*p.His63Asp*) compound heterozygosity cannot be held responsible for clinically significant iron excess so that, in practice, there is no real indication for checking H63D. Exceptionally, other types of compound heterozygosities (involving C282Y as one of the two mutations), or type 4B ferroportin *SLC40A1* mutations can be involved.

Looking for additional potential genetic risk factors (as mentioned above) could also be relevant.

- In a non-caucasian individual, or in the absence of C282Y homozygosity in a caucasian person, specific genetic tests should be performed, at best in the frame of established reference centers. In case of young patients (before the age of 30 years): *HJV* (or *HFE2*), *HAMP*, and *TFR2* mutations ; after age 30: *TFR2* mutations.

#### 2.1.4.2. Phenotype of ferroportin-related iron export deficiency

It should lead to check the *SLC40A1* mutations.

#### 2.1.4.3. Phenotype of HA

Before performing the genetic test, a simple biochemical prerequisite is to ensure the absence of measurable plasma ceruloplasmin(45).

#### 2.1.5. HISTOLOGICAL APPROACH

Classically(46), liver biopsy was performed for: i) proving iron overload (Perls staining) ; ii) quantifying iron overload (histological semi-quantification and/or biochemical determination for liver iron concentration) ; iii) orientating the etiology (hepatocyte versus macrophage iron deposition) ; iv) assessing liver fibrosis, and iv) searching for cofactors of hepatic toxicity, such as features of metabolic syndrome or alcoholism.

### 2.2. OPTIMISING THE DIAGNOSTIC STRATEGY

#### 2.2.1. DIAGNOSING IRON OVERLOAD HAS BECOME A NON INVASIVE APPROACH

Whereas, for a long time, performing a liver biopsy was an indispensable step, it is no more so for the following reasons :

- Proving, quantifying, and assessing indirectly cellular distribution (via the balance between liver and spleen iron deposition) can be accurately achieved by MRI.
- Transient elastography is increasingly performed, as a non invasive surrogate for liver biopsy, to assess liver fibrosis(47).

#### 2.2.2. NEW PROMISING BIOLOGICAL TOOLS ARE EMERGING

- A number of recent biochemical parameters should, in the near future, be more and more applied to investigate disorders of iron metabolism. Among them, the measurements of plasma NTBI, LPI, and possibly hepcidin concentrations.
- The recent advances in molecular biology, especially through next generation sequencing (NGS), are already transforming the genetic diagnosis for iron-related disorders(48). This high throughput approach is a powerful way to identify mutations. However, it raises interpretation issues since it is able to identify a number of new variants of which it is difficult to know whether or not they are harmful.

#### 2.2.3. CLINICAL UTILITY TO PROPOSE THE CONCEPT OF HEPCIDIN-DEPRIVATION SYNDROME (HDS)

The HDS syndrome, observed in all cases of HC (but type A ferroportin disease) and in dyserythropoiesis, can be characterized by the association of increased plasma transferrin saturation, parenchymal (hepatocyte) iron deposition, and absence of splenic iron overload. It exposes to severe complications of iron overload, due to the parenchymal LPI impact.

2.2.4 SETTING UP NATIONAL AND INTERNATIONAL REFERENCE CENTERS AND NETWORKS, for improving the diagnosis of iron overload disorders related to rare HFE or non-HFE mutations, should be part of the health decision-maker policies.

### 3.THERAPEUTIC ASPECTS (Fig.3)

#### 3.1.CURRENTLY AVAILABLE THERAPEUTIC TOOLS AND STRATEGIES

### 3.1.1. VENESECTION THERAPY

3.1.1.1. Rationale. To eliminate excessive stored iron by inducing its release into the blood stream through the recycling effect of repeated erythrocyte withdrawal.

3.1.1.2. Indications : Mainly the hepcidin deprivation syndrome, as present in types 1, 2, 3, and 4B-HC. Residual iron overload after blood stem cell transplantation can also be successfully eliminated by bloodletting(49).

3.1.1.3. Method. The initial phase (called induction phase) consists of iron overload elimination. Venesections, of approximately 7ml/kg body weight (without exceeding 550mL)(50), should be done on a regular weekly basis.

3.1.1.4. Tolerance monitoring. It is evaluated both on clinical data (general tolerance ; blood pressure) and on hemoglobin (Hb) levels which should not decrease below 11g/dL or by more than 2g compare to baseline levels.

3.1.1.5. Efficacy follow-up. It is based on plasma ferritin levels, checked every month until the values reach the upper normal limits, and every two weeks thereafter, until the final goal of ferritinemia levels close to 50 µg/L is reached. It should be kept in mind that transferrin saturation levels remain high until the very end of the induction phase, so that this parameter is not appropriate for evaluating the gradual decrease of iron excess due to the venesections. After iron depletion has been achieved, the maintenance phase starts, lasting, theoretically, for the whole life, and aiming to maintain ferritin levels close to 50 µg/L. It usually consists of one venesection every 2 to 4 months.

3.1.1.6. Results. In HC due to hepcidin deficiency, venesection therapy is globally well tolerated although, in a recent international survey on 210 type HC patients(51), 52% of induction patients and 37% of maintenance patients experienced side effects “always” or “most of the time” after phlebotomy. The overall efficacy to remove iron overload is excellent and many symptoms improve ; however, the joint symptoms may not been improved and even deteriorate or appear under depleting treatment(52, 53).

### 3.1.2. CHELATION

3.1.2.1. Rationale. To use a compound which, after having bound iron at the cellular and/or plasma compartment levels, eliminates the captured iron through the urinary and/or intestinal routes.

3.1.2.2. Indications. Chelation is indicated whenever significant iron overload occurs in the context of chronic anemia, encompassing hemoglobinopathies (mostly major and intermedia thalassemias) and selected cases of myelodysplasia.

3.1.2.3. Methods. Three main compounds are available(54). The oldest one is desferrioxamine or deferoxamine (DFO) (Desferal®). As a hexadentate (one chelator molecule binds one iron atom) it is a powerful and very steady chelator. It is eliminated via the urinary and fecal route. It needs to be administered parenterally and has a very short half-life (20-30 min). Deferiprone (DFP) (Ferriprox®) has been the first oral chelator. It is a bidentate compound (three molecules are needed to bind one iron atom) with a relatively short half-life (1.5-2.5h). Its elimination is essentially through the urinary route. Deferasirox (DFX) (Exjade®) is the most recent oral chelator. It is a tridentate compound (two molecules for binding one iron atom) with a long half-life (10-16h). It is essentially eliminated through the biliary and then fecal route.

3.1.2.4. Tolerance. The major DFO drawback is its way of administration. The chelator is usually administered as a prolonged subcutaneous infusion, using a portable pump, 12 hours a day, at least five days a week, therefore raising important compliance issues, especially in children. DFP requires a three daily dose administration (a liquid formulation has been designed to avoid taking too many tablets) and may induce, exceptionally but unpredictably, agranulocytosis, therefore leading to a systematic monitoring, on a weekly basis, of the blood cell count. DFX presents the advantage of being taken only once a day. It can result in, usually transient, renal failure (creatinine



increase), gastrointestinal symptoms (expected to be lessened by a new formulation), skin rashes, or transaminase increase.

3.1.2.5. Efficacy. All three compounds are efficient. DFP has an interesting efficacy on cardiac iron overload(55). DFX is able to negativate iron balance and to exert beneficial hepatic effects on iron-related hepatic inflammation and fibrosis(56).

### 3.1.3 FAMILY SCREENING (GENETIC DISEASES)

3.1.3.1. General strategy. Family screening is based on the following three main data :

i) the genetic profile that has been identified in the proband and serves as a marker to evaluate genetic predisposition among the relatives, ii) the phenotypic markers, combining plasma transferrin saturation and ferritin levels, and iii) the recessive or dominant mode of transmission of the disease.

3.1.3.2. Differential strategies :

- Quantitative hepcidin deficiency-related HC (types1, 2, or 3 HC). i) Type1 (C282Y/C282Y)-HC(44). Only C282Y homozygosity exposes to the disease *risk*, keeping however in mind that homozygosity does not mean « disease » (due the partial penetrance of the gene). Siblings (genetically explored only after the age of 18) are the primary at risk relatives. However, given the high HFE mutation prevalence, it is recommended to screen genetically also the offspring (after age 18). For the parents, it is usually sufficient to check the phenotypic markers, limiting the genetic study to the cases with phenotypic suggestion of established iron excess. ii) Type2 –HC. Being exceptional, recessive, and juvenile diseases, screening should focus on the siblings (whatever their age). iii) Type3-HC. The screening strategy is close to that of Type1-HC with the differences that, being a rare disease, the offspring are not at risk, and that, since young individuals may be affected, siblings should be explored whatever their age.

- Types 4A and B – HC. The screening procedure should consider both the identical dominant mode of transmission, and the distinct phenotypic expression of these two types of ferroportin diseases.

### 3.2. OPTIMISING THE THERAPEUTIC STRATEGY

#### 3.2.1. CURATIVE ASPECTS : WAYS TO IMPROVE IRON OVERLOAD REMOVAL

3.2.1.1. Hepcidin-deprivation HC (types 1, 2, 3, 4B-HC) : In the most severe forms of adult or juvenile HC(57), combining venesections and chelation may be an option. The interest of adjoining new forms of therapies, such as hepcidin induction(58) (excluding of course type 4B-HC which involves hepcidin refractoriness) through exogenous administration(59) or endogenous stimulation of hepcidin (via, for instance, stimulation of the BMP-SMAD signalling pathway), needs further investigation.

3.2.1.2. Ferroportin iron export deficiency-HC (type 4A-HC) : How to stimulate this ferroportin property remains a research objective.

3.2.1.3. Transfusional and dyserythropoietic iron overload : With the available chelators, there are two major trends for ameliorating chelation efficacy. One is to increase the doses with the risk of increasing side effects. The other option is to combine chelators either by simultaneous or sequential administration, and promising results have already been obtained(60, 61). A further strategy is to find new chelators, closer to an ideal profile in terms of efficacy, tolerance and cost. Innovative therapies could resort to apotransferrin supplementation(62) or TMPRSS6 inhibition(63).

3.2.1.4. Whether antioxidant therapy could be a significant favorable adjunct remains to be clinically fully demonstrated(64-66).

#### 3.2.2 PREVENTIVE ASPECTS

##### 3.2.2.1 GENETIC DISEASES (Type 1-HC)

- Practical measures should be reinforced to diagnose and treat the disease as early as possible. They include : i) Increased awareness of the disease among the medical community, general population and health authorities, with the critical help of patient associations ; ii) Developing family screening centers which are necessary when considering the frequent geographical scattering of the relatives ; iii) Setting up national and international reference centers in the field of genetic iron overload disorders ; iv) As to the mass screening of caucasian populations, it remains debated but seems a reasonable preventive goal in view of the following data : HFE-HC is a frequent disease, with a long clinically asymptomatic phase, an easy and non invasive diagnosis, and furthermore an effective treatment.

- From the medical and scientific viewpoint, preventive improvements should come from two main directions : one is to identify *predictive* markers of phenotypic expression, another one is to investigate the interest of hepcidin supplementation, especially for preventing iron overload development in early diagnosed (and potentially expressing) homozygotes, and to avoid iron overload reconstitution after the end of the induction phase.

#### 3.2.2.2. ACQUIRED DISEASES (TRANSFUSIONAL IRON OVERLOAD)

Associating plasma iron, transferrin saturation and ferritin determination together with MRI assessment of iron overload, should permit to start the chelation therapy as early as possible.

In conclusion, the iron overload field concerns a wide variety of potentially severe diseases of genetic or acquired origin. Key advances in the knowledge of iron metabolism joined to major biological, pharmacological and technological improvements have considerably improved the diagnostic and therapeutic approaches of these diseases. The active ongoing

research in these domains is opening highly promising roads for continuous amelioration of patient care.

#### EXPERT COMMENTARY

In contrast to iron deficiency which is widely recognized as a major health problem, iron overload disorders remain poorly known to the general population and even to the medical community, probably largely due to the fact that iron remains, in our collective unconscious, synonymous with strength and good health. It is therefore important to disseminate informations concerning the major recent advances in the diagnostic and therapeutic management of these iron overload disorders which, whatever their genetic or acquired origin, may severely impact the quality of life and life expectancy of the affected adults or children.

## FIVE-YEAR VIEW

- For genetic iron overload, especially related to hepcidin deficiency, many new variants will be discovered and it is hoped that some of them will permit to predict the phenotypic expression of the genetic predisposition. This prediction should ideally concern not only the amount of iron overload expected in the absence of treatment but the target organs likely to be affected in case of iron excess. An earlier diagnosis, thanks to increase awareness of the diseases and to extended phenotypic screening (based on plasma iron and/or transferrin saturation and/or ferritin levels), should, together with novel predictive markers of phenotypic expression, permit to initiate preventive therapy, ideally consisting of hepcidin supplementation.
- For transfusional iron overload, the diagnosis should benefit from greater accessibility to MRI permitting to start the treatment as soon as possible. One cannot exclude that novel oral iron chelators will be developed during this period but defining the best combined strategies with the existing chelators appear the most promising orientation. Whether hepcidin supplementation will have a significant role in the therapeutic approach of this type of iron overload is a very stimulating perspective.

## KEY ISSUES

- Iron is crucial for life but iron overload, like iron deficiency, can be severely deleterious for the human body.
- Iron overload can be of genetic or acquired origin. Hemochromatosis and transfusional iron overload are the respective archetypes of the corresponding diseases.
- Hemochromatosis is due to either increased iron entry in the cells or decreased cellular iron egress. Heparin and (usual) ferroportin deficiencies are the respective mechanisms underlying these two types of hemochromatosis.
- Iron overload due to hepcidin deficiency is characterized by high plasma iron and transferrin saturation together with parenchymal (essentially hepatocyte) iron deposition.
- Plasma NTBI (non-transferrin bound iron) and its LPI (labile plasma iron) component are new iron species, responsible for iron overload and iron toxicity, respectively.
- MRI (magnetic resonance imaging) has become the non-invasive technique of reference for assessing hepatic (and splenic) iron load.
- Next generation sequencing is transforming the genetic diagnostic strategy and should be handled by expert centers.
- Venesection therapy remains the basis for removing iron excess in diseases related to hepcidin deficiency and could be, in the future, associated with (or replaced by) hepcidin supplementation.
- Chelation therapy has greatly benefited from the introduction of oral chelators. Combining different chelators represents a promising therapeutic perspective.

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FIGURE 1. Iron overload disease spectrum. BMSCT : bone marrow stem cell transplantation.

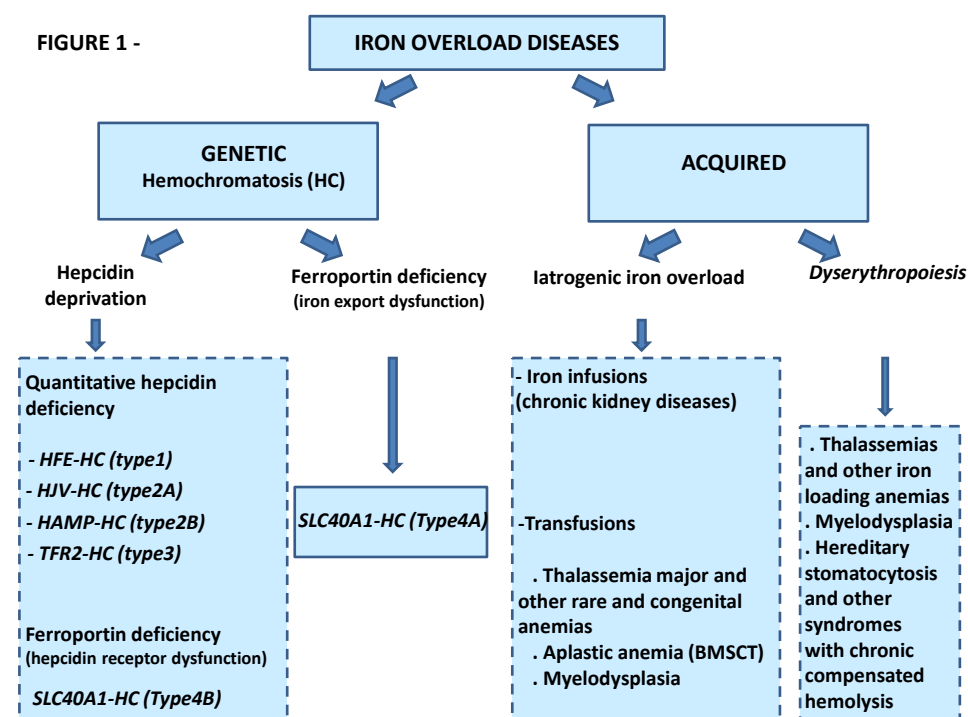


FIGURE 2. Schematic strategy for the diagnosis of iron overload diseases. M: men ; W: women. Black liver or spleen : heavy iron overload (appearing black due to very low MRI signal) ; Grey liver: moderate iron overload ; White liver or spleen: no iron overload (normal MRI signal). \* Clinical symptoms mean: chronic fatigue, joint and bone symptoms, hepatic, pancreatic or cardiac symptoms. \*\* after having ruled out common causes of hyperferritinemia (metabolic syndrome, inflammation, cytotoxicity, alcoholism).

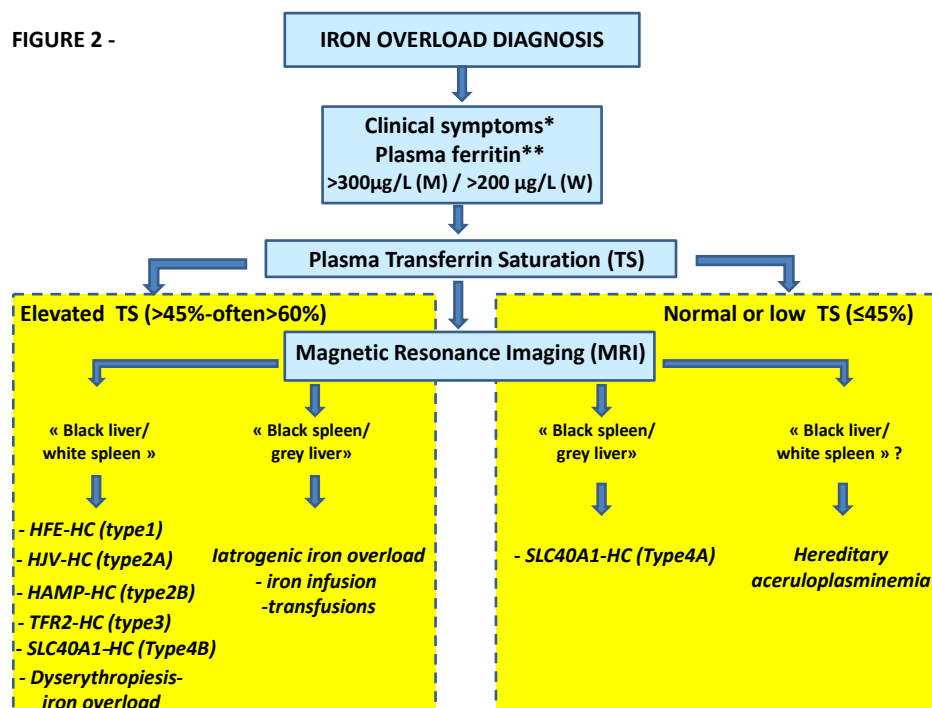


FIGURE 3. Schematic strategy for the treatment of iron overload diseases.

