Les colloques de l'Institut Servier

Preventing and treating iron overload

Oliver Rosmorduc, Paris

Springer Healthcare

Notes are linked to the references page.

Based on three examples of iron overload — respectively of genetic, metabolic and haematological aetiology — this paper will discuss whether it is possible to personalise patient care for this condition.

IRON OVERLOAD OF GENETIC AETIOLOGY

Factors that affect the clinical expression of iron overload

Mutations responsible

The various forms of iron overload can be classified according to the pathogenic mechanism.

Those in the first group are caused by genetic abnormalities that result in an increased intracellular iron entry. These affect HFE (6p21-R), transferrin receptor 2 (7q22-R), hepcidin (19q13-R) or haemojuvelin (1p21-R). Very early onset (in the first or second decade of life) is associated with mutation of the genes that code for hepcidin and haemojuvelin; later onset (in the third and fourth decades) is associated with HFE and the gene for transferrin receptor 2.

A second group includes abnormalities of ferroportin (2q32-D), which results in diminished efflux of iron out of cells and does not tend to manifest until even later, in the fourth or fifth decade.

The clinical picture of iron overload is therefore disparate, with widely varying ages of onset depending on the causal mutation. Purely genetic factors therefore have major consequences when it comes to both screening and the clinical impact of iron overload.

Associated mutations

Hepcidin, a peptide secreted by the liver in response to iron overload, plays a central role in all the genetic abnormalities. Its function is to inhibit the intestinal absorption of iron and stimulate its storage in macrophages. Mutated hepcidin has been found in various cases of juvenile familial haemochromatosis.[1] From a physiopathological point of view, recent work[2, 3] has shown that hepcidin is controlled by haemojuvelin and—probably less directly—by the *HFE* protein, which explains the different phenotypes seen (due to mutations in the two corresponding genes). French studies have shown that heterozygosity at the hepcidin or haemojuvelin loci can underlie a more severe phenotype of *HFE*-related haemochromatosis.[4, 5]

A French team recently showed a link between severe iron overload and the GNPAT p D519G variant in homozygous C282Y haemochromatosis.[6] Some families carrying the homozygous C282Y genotype will suffer from severe iron overload whereas it will be relatively mild in others. In C282Y homozygotes, severe iron overload was associated with the GNPAT p D519G variant in 70–80% of cases, and this variant was never found in C282Y homozygotes without any iron overload. The allele frequency of GNPAT p D519G is 20.6%. In addition, the investigators showed that inhibiting GNPAT with a small interfering RNA (siRNA) results in almost complete suppression of hepcidin: hepcidin—already low in homozygous C282Y haemochromatosis—is further reduced if GNPAT is inhibited.

Understanding the roles of these modulators of iron overload is of great prognostic value and ought to make it possible to select patients who are most at risk of developing a severe, complicated or early-onset form.

Penetrance of homozygous C282Y

The penetrance of the *HFE* C282Y mutation is the third factor that determines the phenotype.
The C282Y mutation is a typical abnormality in haemochromatosis, which is always associated with low hepcidin. In the first cohort studied, the clinical penetrance of the disease seemed to be high. In 1997, Adams et al. found that only 30% of homozygotes showed no signs.[7]

Other studies have shown that while biochemical penetrance is high, clinical penetrance is far lower. An American study by Beutler et al. on more than 41,000 subjects found a clinical penetrance of just 13%.[8] Other genotypes, in particular C282Y/H63D, have even lower penetrance, with fewer than 5% of carriers developing significant iron overload.[9]

Non-genetic factors that affect iron absorption

The clinical expression of HFE haemochromatosis seems to depend on a large number of environmental, pharmacological and physiopathological factors, which modulate the intestinal absorption of iron and therefore account for variability in the degree and age of onset of the overload. This will furthermore have impact on diagnosis and treatment.

The following factors have been shown to be important: the quantity and nature of dietary iron (ferrous, ferric, haem, ferritin, haemosiderin); reducing agents such as vitamin C and chelating agents such as tannins, polyphenols, tetracyclines and hydroxycarbamide; the volume of fluid absorbed; substances that affect iron transport in enterocytes and cardiac muscle cells such as nifedipine; factors that affect the solubility of iron ions; changes in gastric pH; modulators of the iron transporter such as divalent metal transporter 1 (DMT1), ferroportin and transferrin or hepcidin; gastrectomy; inflammation due to infection; age; exogenous iron sources.

It has been shown that long-term treatment with a proton pump inhibitor (PPI) induces a reduction in extracellular and endosomal ferrireductase activities and therefore in the rate of iron absorption.[10] A recent study showed that taking a PPI made it possible to cut down the number of bleeds required by patients with hereditary haemochromatosis.[11]

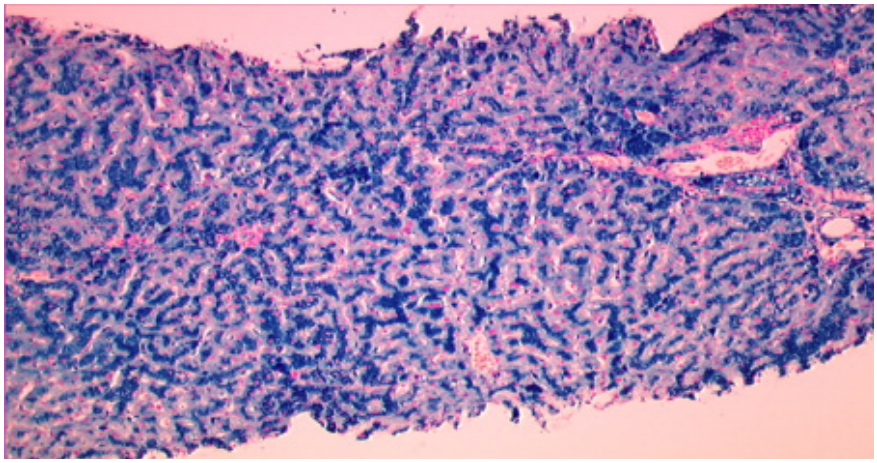
Managing iron overload in haemochromatosis

Diagnosing iron overload

The presence of iron overload is confirmed either by histology, specifically with Perls staining of liver biopsy sections (Figure 1.), or by magnetic resonance imaging (MRI). It is generally accepted that there may be primary or secondary haemochromatosis if the overload is greater than 125 µmoles of iron per gram of liver tissue (7 mg/g). Patients with hereditary haemochromatosis are at risk of hepatic fibrosis or cirrhosis if the load exceeds 331 µmoles of iron per gram of liver tissue (18 mg/g).[12] Hepatic overload should be diagnosed before

such levels are reached, i.e. levels associated with serious or complicated cirrhosis or even hepatocellular carcinoma.

Figure 1. Hepatic iron overload in haemochromatosis: Perls staining of liver biopsy material



Guidelines

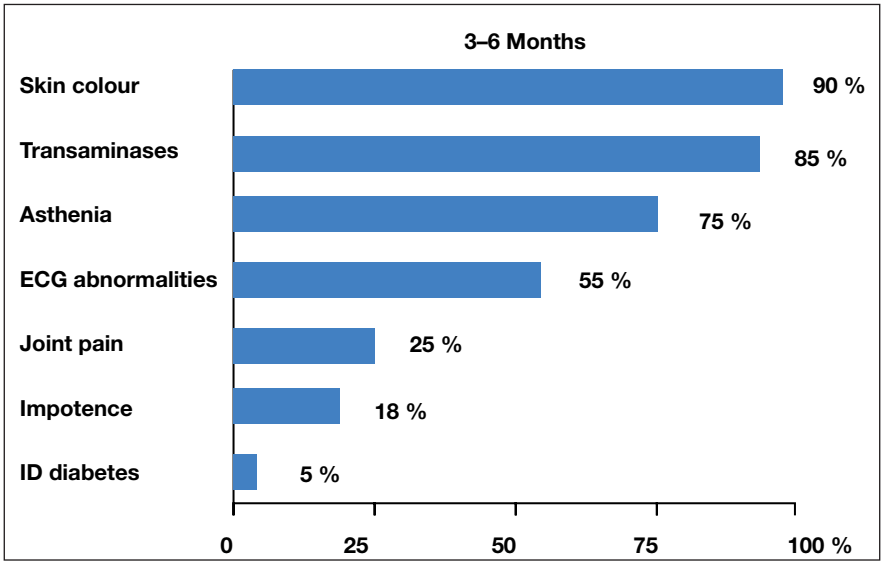
The 2010 guidelines of the European Association for the Study of the Liver (EASL)[13] on managing HFE haemochromatosis recommend establishing a programme of bloodletting if there is iron overload. Simple annual surveillance is recommended in C282Y homozygotes with no sign of iron overload, with treatment started if the blood ferritin level is elevated. Complications (hepatic cirrhosis, diabetes, joint involvement, hypogonadism, porphyria cutanea tarda) should be addressed whether or not haemochromatosis is the underlying cause and whether or not bloodletting has relieved the symptoms.

The 2005 guidelines of the Haute autorité de santé (French Supreme Health Authority)[14] on managing HFE haemochromatosis organise care on the basis of a staging system going from 0 (no symptoms, transferrin saturation (CS-Tf) <45%, ferritinaemia normal) to 4 (clinical manifestations, CS-Tf >45%, hyperferritinaemia, complications). Surveillance only (interview, physical examination, ferritinaemia and CS-Tf) is indicated at stage 0 (once every three years) and stage 1 (once a year). At stage 2, hyperferritinaemia justifies therapeutic bloodletting until a target blood ferritin concentration of 50 µg/l is attained. The frequency of maintenance bleeds varies: every two, three or four months, depending on the case.

Efficacy of bloodletting

Serial bloodletting can bring a number of encouraging benefits, with improvements in pigmentation, transaminase activities, asthenia and ECG abnormalities the most common. Improvement in joint pain is much rarer and there is hardly ever any decrease in the severity of insulin-dependent diabetes or reversal of impotence, once it has developed (Figure 2).[15] Bloodletting has a beneficial effect on the hepatic fibrosis which can regress but not in patients with high gammaglobulin and prothrombin levels or a low platelet count (indicating advanced cirrhosis).[16]

Figure 2. Efficacy of therapeutic bloodletting[15]



The usefulness of bloodletting in patients with C282Y haemochromatosis and a moderately elevated blood ferritin concentration is more debatable. An Australian cohort study showed that at least 28% of male C282Y homozygotes and 1% of female homozygotes develop severe iron overload-related complications (cirrhosis, diabetes or cardiomyopathy).[17] However, the same group showed that C282Y homozygotes with ferritin levels of below 1000 µg/l have a low risk of developing any of the signs or symptoms of haemochromatosis.[18] A single-masked trial currently under way is comparing the progress of patients with moderate ferritinaemia (between 300 and 1000 µg/l) treated by either erythrocytapheresis (active treatment) or plasmapheresis (control). The results should establish whether bloodletting is indicated in this population.

Chelating agents

Agents that chelate iron ions are useful in patients who are unsuitable candidates for phlebotomy because of poor tolerance, inaccessible veins, anaemia or severe heart disease.

Deferasirox (DFX) was tested in a French study on patients with haemochromatosis. After treatment with DFX at a dosage of 5, 10 or 15 mg/kg/day for 48 weeks, the median blood ferritin concentration was reduced by 63.5%, 74.8% and 74.1%, respectively. The median serum ferritin concentration was below 250 µg/l in all groups. Most adverse reactions were dose-dependent, the most frequent being diarrhoea, headache and nausea. The investigators concluded that a dosage of 10 mg/kg DFX a day is the best compromise in terms of efficacy and safety in this patient population.[19]

IRON OVERLOAD OF METABOLIC AETIOLOGY

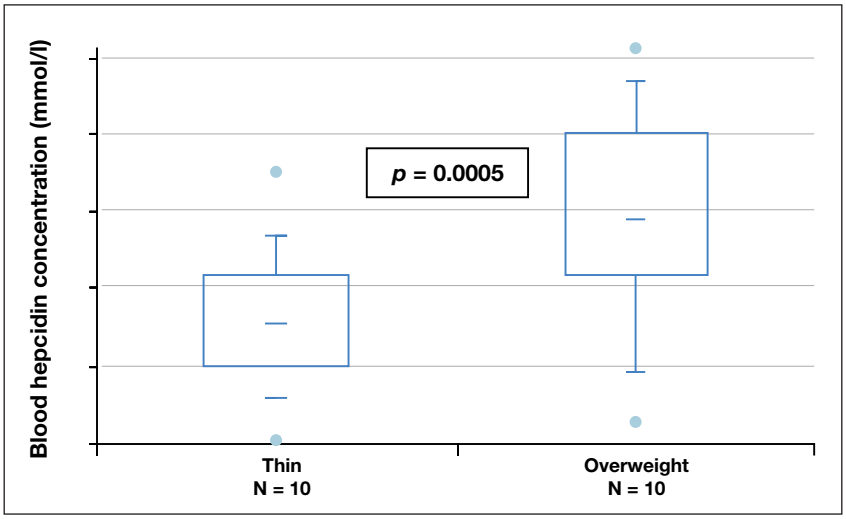
A combination of high blood ferritin and low transferrin saturation coefficient points to primary haemochromatosis with a different aetiology. In this situation, mild hepatic iron overload in MRI suggests hepatosiderosis caused by metabolic dysfunction; high hepatic overload would suggest abnormal ferroportin or aceruleoplasminaemia; and no hepatic overload suggests hyperferritinaemia-cataract syndrome or Gaucher's disease.

Managing hepatic iron overload due to abnormal ferroportin is similar to that of haemochromatosis, depending on bloodletting with surveillance of haemoglobin and ferritin, with a target ferritinaemia of 30–50 µg/l.

The management of dysmetabolic syndromes also relies on bloodletting with surveillance of haemoglobin and blood ferritin coupled with treatment of the metabolic problem. One study[20] showed that in female C282Y homozygotes, a body mass index (BMI) of 28 kg/m2 or over is associated with a lower efficiency of iron

extraction by phlebotomy. High BMI also seems to affect the phenotypic expression of C282Y homozygosity, probably due to high levels of hepcidin in the bloodstream (Figure 3.).

Figure 3. Iron overload in obese, female C282Y homozygotes: role of hepcidin[20]



In non-alcoholic hepatic steatosis (NASH), reducing ferritin by bloodletting does not bring about any improvement in liver function enzyme activities, hepatic steatosis or insulin resistance.[21] Nor did bloodletting have any effect on the liver inflammation of NASH.[22]

In patients with dysmetabolic hypersiderosis, extracting iron by bloodletting (ferritinaemia ≤50 µg/l) does not improve metabolic or hepatic parameters and is not as well tolerated as would be expected.[6]

IRON OVERLOAD IN HAEMOGLOBINOPATHY

Iron overload associated with intestinal absorption is exacerbated by blood transfusion in patients with thalassaemia. There is a relationship between hepatic and cardiac iron overload with a concomitant elevation in cardiovascular risk.[12]

The various recommended therapeutic strategies are based on ferritinaemia, the hepatic iron concentration (HIC) as estimated by MRI, the number of blood transfusions and the heart MRI picture (T2*). As a rule, chelating agents are prescribed after the transfusion of 10–20 units of packed erythrocytes, when ferritin goes above 1000 µg/l or when the HIC is over 7 mg/g (because of a risk of cardiac mortality if the HIC is above 12 mg/g).

Deferoxamine (DFO) is recommended for moderate overload. If the patient is intolerant of DFO, DFX is recommended and if that too is poorly tolerated, then deferiprone (DFP). Intensive DFO treatment or a combination therapy with both DFO and DFP is recommended for severe overload. In patients with cardiac involvement as detected by MRI (T2* >20 ms) DFO or DFP are more suitable than DFX; if T2* is between 10 and 20 ms, DFO plus DFP is recommended; and if T2* <10 ms, intensive DFO plus DFP is recommended.

THERAPEUTIC PERSPECTIVES

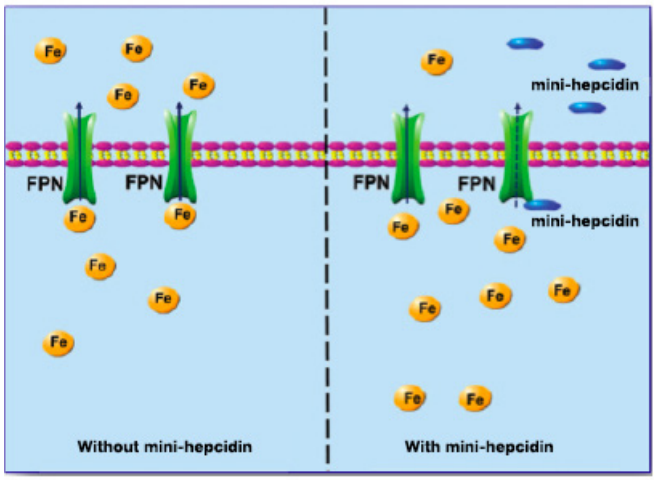
Current chelating agents are much improved in terms of presentation and combination possibilities, including with agents that rechannel iron into haematopoiesis. The newer chelating agents are also less nephrotoxic.

In parallel, it would be useful to be able to cut down requirements for blood transfusion. In this perspective, compounds that target the enzyme JAK2 seem to constitute a promising approach in the treatment of haema-

topoietic disorders. Similarly, sotatercept, an activin receptor type 2A fusion protein, reduces the need for transfusion in patients with beta-thalassaemia.

Hepcidin is obviously an ideal target. Hepcidin inducers—Tmprss6 antagonists and BMP6 agonists—are currently in development.
Substances that can induce the breakdown of ferroportin or reduce its concentration are also in development.
A major research effort is going into developing drugs derived from hepcidin to treat disorders associated with low blood hepcidin levels (see Figure 4.).[23]

Figure 4. Mini-hepcidins and other hepcidin inducers[23]



Hepcidin derivatives (mini-hepcidins) can be used to treat hypohepcidinaemia. Other avenues are currently being evaluated with PR65 and PR73SH (breakdown of ferroportin in vitro and in vivo), genistein (isoflavone, hepcidin induction), progesterone and mifepristone (reduction of ferroportin), BMP6 agonists (hepcidin induction). FPN, ferroportin. © J Liu

CONCLUSION

Surveillance of iron overload and the initiation of treatment could be personalised on the basis of individual risk factors.
Treating severe iron overload will prevent complications, notably hepatic, endocrine and cardiac complications.
Maintenance treatment can prevent relapse. In this respect, novel therapeutic approaches are being investigated, notably oral drugs.
There is not currently any prophylactic treatment modality validated in the absence of significant, documented iron overload.

BIBLIOGRAPHIE *(les références soulignées renvoient aux abstracts correspondants sur pub Med)*

1. Roetto A, Daraio F, Porporato P, et al. Screening hepcidin for mutations in juvenile hemochromatosis: identification of a new mutation (C70R). Blood 2004;103:2407-9.
2. Huang FW, Pinkus JL, Pinkus GS, Fleming MD, Andrews NC. A mouse model of juvenile hemochromatosis. J Clin Invest 2005;115:2187-91.
3. Niederkofler V, Salie R, Arber S. Hemojuvelin is essential for dietary iron sensing, and its mutation leads to severe iron overload. J Clin Invest 2005;115:2180-6.
4. Jacolot S, Le Gac G, Scotet V, Quere I, Mura C, Ferec C. HAMP as a modifier gene that increases the phenotypic expression of the HFE pC282Y homozygous genotype. Blood 2004;103:2835-40.
5. Le Gac G, Scotet V, Ka C, et al. The recently identified type 2A juvenile haemochromatosis gene (HJV), a second candidate modifier of the C282Y homozygous phenotype. Hum Mol Genet 2004;13:1913-8.
6. Lainé F, Ruivard M, Loustaud-Ratti V, et al. Metabolic and hepatic effects of bloodletting in dysmetabolic iron overload syndrome: A randomized controlled study in 274 patients. Hepatology 2017;65:465-74.

7. Adams PC, Deugnier Y, Moirand R, Brissot P. The relationship between iron overload, clinical symptoms, and age in 410 patients with genetic hemochromatosis. Hepatology 1997;25:162-6.
8. Beutler E, Felitti V, Koziol J, Ho N, Gelbart T. Penetrance of 845G > A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet 2002;359:211-8.
9. Gurrin LC, Bertalli NA, Dalton GW, et al. HFE C282Y/H63D compound heterozygotes are at low risk of hemochromatosis-related morbidity. Hepatology 2009;50:94-101.
10. Handa P, Kowdley KV. A Proton Pump Inhibitor a Day Keeps the Iron Away. Clin Gastroenterol Hepatol 2016;14:153-5.
11. van Aerts RM, van Deursen CT, Koek GH. Proton Pump Inhibitors Reduce the Frequency of Phlebotomy in Patients With Hereditary Hemochromatosis. Clin Gastroenterol Hepatol 2016;14:147-52.
12. Rostoker G, Vaziri ND, Fishbane S. Iatrogenic Iron Overload in Dialysis Patients at the Beginning of the 21st Century. Drugs 2016;76:741-57.
13. European Association For The Study Of The Liver. EASL clinical practice guidelines for HFE hemochromatosis. J Hepatol 2010;53:3-22.
14. HAS. Prise en charge de l'hémochromatose liée au gène HFE (hémochromatose de type 1). Synthèse des recommandations. Juillet 2005.
15. Niederau C, Fischer R, Pürschel A, Stremmel W, Häussinger D, Strohmeyer G. Long-term survival in patients with hereditary hemochromatosis. Gastroenterology 1996;110:1107-19.
16. Bassett ML, Halliday JW, Powell LW. Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis. Hepatology 1986;6:24–29.
17. Allen KJ, Gurrin LC, Constantine CC, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. N Engl J Med 2008;358:221-30.
18. Allen KJ, Bertalli NA, Osborne NJ, et al. HFE Cys282Tyr homozygotes with serum ferritin concentrations below 1000 microg/L are at low risk of hemochromatosis. Hepatology 2010;52:925-33.
19. Phatak P, Brissot P, Wurster M, et al. A phase 1/2, dose-escalation trial of deferasirox for the treatment of iron overload in HFE-related hereditary hemochromatosis. Hepatology 2010;52:1671-779.
20. Desgrippes R, Lainé F, Morcet J, et al. Decreased iron burden in overweight C282Y homozygous women: Putative role of increased hepcidin production. Hepatology 2013;57:1784-92.
21. Adams LA, Crawford DH, Stuart K, et al. The impact of phlebotomy in nonalcoholic fatty liver disease: A prospective, randomized, controlled trial. Hepatology 2015;61:1555-64.
22. Beaton MD, Chakrabarti S, Adams PC. Inflammation is not the cause of an elevated serum ferritin in non-alcoholic fatty liver disease. Ann Hepatol 2014;13:353-6.
23. Liu J, Sun B, Yin H, Liu S. Hepcidin: A Promising Therapeutic Target for Iron Disorders: A Systematic Review. Medicine (Baltimore) 2016;95:e3150.