Hyperferritinemia and non-HFE hemochromatosis: differential diagnosis and workup

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Abstract

Hyperferritinemia is a common reason for referral to a hepatogastroenterologist. The most frequent causes are not associated with iron overload (e.g. inflammatory diseases, alcohol abuse, metabolic syndrome, etc.). However, hyperferritinemia can also be caused by a genetic variant in one of the iron regulatory genes, called hereditary hemochromatosis, often but not always associated with iron overload. A variation in the human Hemostatic Iron Regulator protein (HFE) gene is the most common genotype, but many other variants have been described.

In this paper we discuss two cases of rare hyperferritinemia associated disorders, ferroportin disease and hyperferritinemia-cataract syndrome. We also propose an algorithm for evaluating hyperferritinemia, facilitating a correct diagnosis and preventing potentially unnecessary examinations and therapeutic actions. (Acta gastroenterol. belg., **2023,** 86, **356-359**).

Keywords: iron overload;, ferroportin disease, hereditary hyperferritinemia-cataract syndrome

Introduction

Iron plays an essential role in the human body. Deficiency can lead to anemia, impairment of the immune system and cognitive dysfunction (1). On the other hand, iron overload can be toxic for human cells. The regulation involves iron regulatory proteins (IRP), binding to iron- responsive elements (IRE); as well as a delicate balance of iron absorption, recycling and loss. The most relevant proteins to discuss are transferrin, ferroportin, ferritin and hepcidin. Transferrin is a blood plasma iron transporter and is able to bind iron ions, which it acquires from ferroportin. Ferroportin is a cellular exporter of iron, predominantly found on the basolateral surface of duodenal enterocytes and on the membrane of macrophages, allowing iron absorption and recycling. Its expression is inhibited by hepcidin. Ferritin is a large cellular iron storage protein, preventing the catalyzation of free radical formation. Iron storage primarily happens in the reticulo-endothelial cells and hepatocytes. Hyperferritinemia causes the ferritin proteins to aggregate. These aggregates are broken down to hemosiderin, which slowly releases its iron (2).

Hyperferritinemia is a common reason for referral to a hepatogastroenterologist. The most frequent causes of hyperferritinemia are inflammatory disorders (including neoplasia), hepatitis, alcohol abuse, metabolic associated fatty liver disease (MAFLD), metabolic syndrome and acquired iron overload (iron use or blood transfusions). Hyperferritinemia can also be caused by a genetic variant in one of the iron regulatory genes, called hereditary hemochromatosis. A variation in the human Hemostatic Iron Regulator protein (HFE) gene is the most common genotype, but many other variants have been described.

When the most common causes have been ruled out, hepatogastroenterologists should include rare causes of hyperferritinemia in the differential diagnosis, since accurate diagnosis can avoid unnecessary examinations, interventions and phlebotomies.

In this paper we present two cases of rare hyperferritinemia associated disorders in order to increase awareness in your gastroenterologist daily practice.

Case 1

A forty-five year old male patient presented with hyperferritinemia (1624 µg/L) and a transferrin saturation of 36% at the outpatient clinic. Additional blood examination revealed normal liver function tests and CRP. Furthermore, no inflammatory disorders, features of metabolic syndrome or alcohol abuse were present. Gene sequencing showed no C282Y gene variant. A magnetic resonance imaging (MRI) scan of the upper abdomen showed clear iron overload in the liver, not in the spleen. A liver biopsy was performed and microscopic examination revealed moderate liver steatosis and iron overload with a clear gradient pattern, favoring the Kupffer cells. Because of the typical iron overload gradient and radiological features specific genetic analysis was performed, confirming a heterozygous p.Arg178Gln variant of the SLC40A1 gene, a published pathogenic variant (3, 4) and therefore diagnostic for Ferroportin-associated hereditary hemochromatosis. Monthly phlebotomies were started and were well tolerated.

Case 2

A thirty-five year old female patient presented with chronic hyperferritinemia (1600 µg/L) and a transferrin saturation of 46% at the outpatient clinic. Additional blood examination revealed normal liver function tests

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Table 1. — **Summery table, comparing features of HH1, ferroportin disease and HHCS**

	HH1	Ferroportin disease (hepatic-type)	HHCS
Prevalence OMIM	1/220-250 (10) OMIM #235200	< 1/1,000,000 (11) OMIM #606069	1/200,000 (12) OMIM #600886
Clinical presentation	- Asymptomatic - Fatigue, skin hyperpigmentation, arthralgia, impotence - Usually present from the 4th to 5th life decade: diabetes mellitus, liver cirrhosis	- Asymptomatic - Fatigue, skin hyperpigmentation, arthralgia, impotence, arrhythmias - Usually present from the 3rd to 4th life decade (13): diabetes mellitus, liver cirrhosis	- None - Decreased vision / early onset cataract (familial)
Biochemical	- Hyperferritinemia - Elevated transferrin saturation - Liver function abnormalities possible	- Hyperferritinemia - Elevated transferrin saturation - Liver function abnormalities possible	- Hyperferritinemia (often ≥ 1000 mg/L) - Normal transferrin saturation
Imaging (MRI)	- Signs of hepatic iron overload	- Signs of hepatic iron overload	Normal
Liver biopsy	- A degree of iron overload, primarily in the hepatocytes - Steatosis, cirrhosis	- A degree of iron overload, primarily in the Kupffer cells - Steatosis, cirrhosis	Normal
Genetic analysis	- HFE gene mutation - Autosomal recessive, variable penetrance	- SLC40A1 gene mutation (gain-of-function) - Autosomal dominant, higher penetrance (13)	L-ferritin IRE gene mutation (> 25 known mutations) Autosomal dominant (5)
Treatment	- Therapeutic phlebotomy - Avoid iron and vitamin C supplementation	- Cautious therapeutic phlebotomy - Avoid iron and vitamin C supplementation	- No therapeutic phlebotomy - Cataract surgery if necessary
Follow-up	Hepatological	Hepatological	Ophthalmological
Familial screening	Analysis of iron tests and genetic analysis for all first degree family members	Analysis of iron tests and genetic analysis for all first degree family members	Analysis of iron tests for all first degree family members

HH1: hereditary hemochromatosis type 1. HHCS: hereditary hyperferritinemia-cataract syndrome.

and CRP. Furthermore, no inflammatory disorders, features of metabolic syndrome or alcohol abuse were present. The patient and her sister had undergone phlebotomies for several years at the general practitioner's, resulting in severe iron deficiency anemia. Genetic testing showed no C282Y or H63D gene variants.

An MRI scan of the upper abdomen showed no signs of iron overload in the liver nor in the spleen. Additional anamnesis revealed cataract in several relatives. Our patient did not wish additional genetic analysis to be performed, however her sister did. Specific genetic analysis confirmed a heterozygous c.-160A>G variant of the FTL gene, a published pathogenic variant (5, 6) and therefore diagnostic for hereditary hyperferritinemia-cataract syndrome (HHCS). The patient could be reassured, no more phlebotomies were necessary. There is no need for hepatologic follow-up. Ophthalmological follow-up was provided.

Discussion

Hyperferritinemia can be caused by inflammation, alcohol abuse, MAFLD or metabolic syndrome. It can also be a sign of iron overload, acquired or hereditary. The first step is to determine whether the elevated ferritin levels truly represent iron overload. A classic workup consists of a comprehensive patient and family history, a standard blood test including liver function and iron tests; and an ultrasound of the abdomen. When true iron overload is suspected, additional testing is warranted; genetic testing, MRI scan of the upper abdomen and rarely a liver biopsy might be indicated (figure 1). According to the most recent EASL guidelines, MRI scan should be performed in patients with biochemical iron overload without homozygosity for p.C282Y or in the presence of additional risk factors (7).

Hereditary hemochromatosis type 1 (HH1) is one of the most common genetic disorders in Europe and the most common genetic cause of hyperferritinemia. It is an autosomal recessive disorder with low disease penetrance, caused by a variation in the HFE gene. C282Y and H63D variations are the most common. Laboratory findings show elevated transferrin saturation and ferritin levels; and sometimes liver function test abnormalities. The diagnosis is confirmed when gene sequencing shows biallelic HFE variants. An MRI scan can be used to quantify liver iron. Liver biopsy is not essential for the diagnosis and should be reserved to evaluate hepatic cirrhosis or fibrosis (8).

Genetic analysis excluded this disorder in the two described cases.

The first case described a patient with hepatic-type Ferroportin disease. Additional genetic testing was performed because of the presence of iron overload.

Ferroportin disease or hereditary hemochromatosis type 4 (HH4) is a rare genetic condition. Two phenotypes have been described. The macrophage-type or classical disease is caused by loss-of-function mutations, resulting in a ferroportin molecule that is not exporting iron properly. Aside from hyperferritinemia, these patients

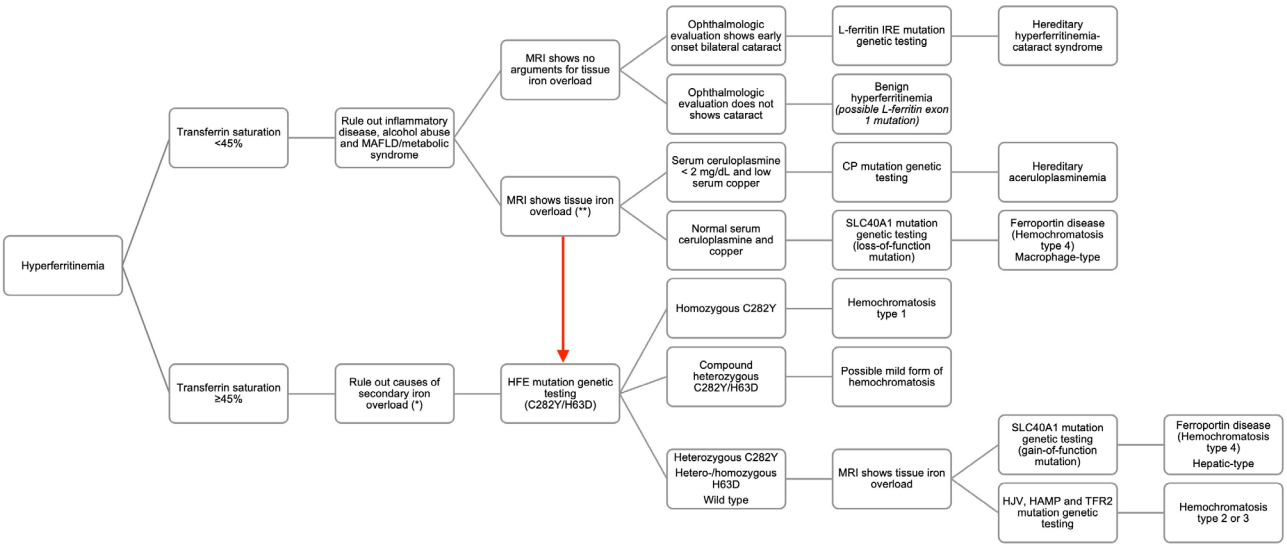

Figure 1 – Flowchart differential diagnosis hyperferritinemia (4, 7, 9). *Iron use, multiple blood transfusions, hematological disorders (e.g. thalassemia, myelodysplasia, …) (7). ***Splenic iron overload is very suggestive of ferroportin disease macrophage-type (4). MAFLD: metabolic associated fatty liver disease. MRI: magnetic resonance imaging. When in doubt; repeat iron test after 3-6 months, consider liver biopsy or specific genetic testing.*

have normal to reduced transferrin saturation and mild anemia. The hepatic-type or non-classical disease is caused by gain-of- function mutations, resulting in a ferroportin molecule that is resistant to hepcidin, in turn resulting in excess iron exportation. These patients have an elevated transferrin saturation and hepatic iron overload, presenting similar to HH1. Anatomopathological examination of liver tissue primarily shows iron overload in the reticulo-endothelial system (e.g. Kupffer cells) as opposed to in the hepatocytes, like seen in HH1. Patients are at risk for the same organ complications as patients suffering from HH1, though usually the disease is milder, as this pattern of iron overload is better tolerated and less fibrogenic than parenchymal cell iron overload. Therapeutic phlebotomy is indicated, however because of poor tolerance a lower frequency should be applied (4).

The second patient was diagnosed with HHCS, first described by Girelli *et al.* (9) and Bonneau *et al.* (5) in 1995. Genetic testing was performed, because of the typical family history of cataract and absolute intolerance of phlebotomies.

HHCS is an autosomal dominant inherited syndrome, characterized by hyperferritinemia with normal transferrin saturation and no arguments for tissue iron overload, in association with early onset bilateral cataract (9).

The responsible gene variants for HHCS, are point mutations and deletions in the highly conserved IRE structure (mRNA), a 5' untranslated region of the light chain or L-ferritin gene on chromosome 19. These variants reduce IRE-IRP binding, resulting in L-ferritin upregulation (9). L-ferritin is an isoferritin. It is responsible for stabilizing the ferritin shell and acts as a catalyst promoting iron oxidation. However, it is not involved in iron uptake (10). Ferritin measured in serum samples mainly contains L subunits. This explains the existence of hyperferritinemia without tissue iron overload. The only organ that is affected by this L-hyperferritinemia is the eye. Lenzhofer *et al.* described a HHCS patient with a 23 to 25-fold increase in aqueous humor ferritin levels versus a control group, resulting in early onset bilateral cataract (11). A long-term observational study showed a slowly progressive opacification of the lens over the years (12).

There is no need for any form of treatment, besides management of ophthalmological symptoms. Therapeutic phlebotomy is not indicated, and can even be hazardous, causing severe symptomatic anemia.

As a result of the increased availability of MRI, the necessity for liver biopsy to determine tissue iron deposition has diminished. As mentioned, a liver biopsy is not required for the diagnosis of HH1. Specific histological abnormalities can help differentiate in cases of non- HFE hemochromatosis and guide specific genetic testing, however liver biopsy is not required for the diagnosis of ferroportin disease or HHCS either (8). The remaining indications are to diagnose hepatic cirrhosis, when there is uncertainty using non-invasive tests; and to stage hepatic fibrosis when ferritin levels are higher than 1000 µg/L, liver enzymes are elevated, elastography is indeterminate or in the presence of hepatomegaly. This ensures appropriate surveillance for hepatocellular carcinoma (7).

We acknowledge this article does not cover all hereditary haemochromatosis syndromes, for example hereditary aceruloplasminemia and juvenile hemochromatosis due to variants in the genes for hemojuvelin (HH2), hepcidin or transferrin receptor 2 (HH3).

Also, we emphasize that the proposed flowchart (figure 1) is a guide for clinicians, not a validated algorithm. When there is a high clinical suspicion, further investigation is required.

Conclusion

In this article we describe two patients presenting with hyperferritinemia. We confirm ferroportin disease in patient number one and HHCS in patient number two. Except for HH1, these two primary iron storage diseases are most prevalent, however often unfamiliar. We discuss (differential) diagnosis and treatment. We propose a flowchart, facilitating a correct diagnosis and preventing potentially unnecessary examinations and therapeutic actions.

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References

1. ABBASPOUR N., HURRELL R., KELISHADI R. Review on iron and its importance for human health. *J Res Med Sci.* 2014, **19**(2):164-74.
2. HENTZE M.W., MUCKENTHALER M.U., GALY B., CAMASCHELLA C. Two to tango: regulation of Mammalian iron metabolism. *Cell*. 2010, **142**(1):24-38.
3. KA C., GUELLEC J., PEPERMANS X., KANNENGIESSER C., GED C., WUYTS W.*, et al.* The SLC40A1 R178Q mutation is a recurrent cause of hemochromatosis and is associated with a novel pathogenic mechanism. *Haematologica*. 2018, **103**(11):1796-805.
4. PIETRANGELO A. Ferroportin disease: pathogenesis, diagnosis and treatment. *Haematologica*. 2017, **102**(12):1972-84.
5. BEAUMONT C., LENEUVE P., DEVAUX I., SCOAZEC J.Y., BERTHIER M., LOISEAU M.N.*, et al.* Mutation in the iron responsive element of the L ferritin mRNA in a family with dominant hyperferritinaemia and cataract. *Nat Genet*. 1995, **11**(4):444-6.
6. LUSCIETI S., TOLLE G., ARANDA J., CAMPOS C.B., RISSE F., MORAN E.*, et al.* Novel mutations in the ferritin-L iron-responsive element that only mildly impair IRP binding cause hereditary hyperferritinaemia cataract syndrome. *Orphanet J Rare Dis*. 2013, **8**:30.
7. EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER. ELECTRONIC ADDRESS E.E.E., EUROPEAN ASSOCIATION FOR THE STUDY OF THE L. EASL Clinical Practice Guidelines on haemochromatosis. *J Hepatol*. 2022, **77**(2):479-502.
8. KOWDLEY K.V., BROWN K.E., AHN J., SUNDARAM V. ACG Clinical Guideline: Hereditary Hemochromatosis. *The American journal of gastroenterology*. 2019, **114**(8):1202-18.
9. GIRELLI D., CORROCHER R., BISCEGLIA L., OLIVIERI O., ZELANTE L., PANOZZO G.*, et al.* Hereditary hyperferritinemia-cataract syndrome caused by a 29-base pair deletion in the iron responsive element of ferritin L-subunit gene. *Blood*. 1997, **90**(5):2084-8.
10. AROSIO P., LEVI S. Cytosolic and mitochondrial ferritins in the regulation of cellular iron homeostasis and oxidative damage. *Biochim Biophys Acta*. 2010, **1800**(8):783-92.
11. LENZHOFER M., SCHROEDL F., TROST A., KASER-EICHBERGER A., WIEDEMANN H., STROHMAIER C.*, et al.* Aqueous humor ferritin in hereditary hyperferritinemia cataract syndrome. *Optom Vis Sci*. 2015, **92**(4 Suppl 1):S40-7.
12. COSENTINO I., ZERI F., SWANN P.G., MAJORE S., RADIO F.C., PALUMBO P.*, et al.* Hyperferritinemia-cataract syndrome: Long-term ophthalmic observations in an Italian family. *Ophthalmic Genet*. 2016, **37**(3):318-22.