Recent advances in genetic iron overload-related disorders

Summary

Serum ferritin is one of the most frequently requested laboratory tests in both primary care and referral settings. Ferritin is a cellular iron storage protein and for that reason serum ferritin is a reliable surrogate marker of body iron stores. Low serum ferritin levels provide absolute evidence of reduced iron stores. However, high serum ferritin levels (hyperferritinemia) are far less specific for systemic iron overload since ferritin is also an acute phase protein and will increase in case of infection, neoplasm and acute or chronic inflammation. Hyperferritinemia is defined as serum ferritin concentrations >200 µg/L in women and >300 µg/L in men, and is found in around 12% of the general population. Since hyperferritinemia is common and often does not reflect iron overload it is a great challenge for physicians to determine its exact cause.

This thesis consists of two parts. In the first part the focus is on the diagnostic difficulties in patients with hyperferritinemia, in the second part on *HFE*-related hemochromatosis.

HFE-related hemochromatosis is a frequent cause of hyperferritinemia, and is associated with iron overload. The most prevalent form is homozygosity for the p.Cys282Tyr variant in the *HFE* gene and this is the most common autosomal recessive, genetic disorder found in Caucasians. It is most commonly seen in populations of Northern European origin, in which the prevalence is close to 1 per 200-250 persons. *HFE*-related hemochromatosis is characterized by low hepcidin levels which result in a persistent iron absorption leading to iron accumulation in the body`s tissues and organs, particularly the liver, pancreas, joints, heart and the skin. Iron accumulation will eventually lead to organ damage resulting in hepatic cirrhosis, primary liver cancer, arthropathy, cardiomyopathy and diabetes mellitus. To maintain a normal life expectancy iron depletion therapy should be started in time in order to prevent iron accumulation and its complications.

Part 1- Understanding and interpreting hyperferritinemia

A frequent cause of hyperferritinemia is non-alcoholic liver disease (NAFLD), the most widespread liver disorder in Western society. In 30% of patients with NAFLD, hyperferritinemia is found, however its origin is a subject of discussion. Prior to starting therapy the etiology of hyperferritinemia should be investigated since iron depletion therapy is not advised in inflammation-related hyperferritinemia nor in patients with the dysmetabolic iron overload syndrome. In **chapter 2** an extensive

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literature search was performed to investigate whether hyperferritinemia in NAFLD is an expression of iron overload or inflammation. It was shown that in the majority of cases hyperferritinemia in NAFLD is related to inflammation. In a smaller group, dysmetabolic iron overload syndrome is found, showing inflammation related hyperferritinemia in combination with mild iron accumulation in the reticuloendothelial cells. In the smallest group, hyperferritinemia in NAFLD is related to genetic disturbances of iron homeostasis such as *HFE*-related hemochromatosis.

Because of the broad etiological spectrum of hyperferritinemia it is a challenge to determine its cause. When clinical examination and additional laboratory tests do not provide a certain diagnosis, the liver iron concentration (LIC) can be used. Previously, the LIC was determined in a liver tissue sample collected via liver biopsy. Liver biopsy however, is an invasive procedure with potentially serious complications and the risk of sample error. For that reason, nowadays, the LIC measured by MRI is used (using a specific iron protocol). The LIC is considered the best method to accurately assess body iron load, since the liver contains ≥70% of the body iron stores. However, there are difficulties with interpreting the LIC with the generally used cut-off value of ≥36 µmol/g. This value appears to be low since often the LIC is found to be increased in hyperferritinemia associated with the dysmetabolic iron overload syndrome and/or alcohol (over)consumption in the absence of major iron overload. Previously, the liver iron index (LII) was introduced to differentiate patients with *HFE*-related hemochromatosis from patients with alcoholic liver disease or heterozygous hemochromatosis mutations. The LII is calculated by dividing the LIC measured with liver biopsy by the age of the patient in years. The rationale behind introducing the factor age is that iron accumulation in *HFE*-related hemochromatosis is a dynamic process gradually increasing in the course of life. On a yearly basis, patients with *HFE*-related hemochromatosis absorb about 1g more iron than the body requires. Since a good correlation was found between the LIC measured by biopsy and by MRI scan, the aim of the study was to investigate if the LII derived from the LIC measured by MRI could also be used to interpret hepatic iron presence (**chapter 3**). A retrospective cohort study was conducted involving hyperferritinemia patients who underwent a MRI according to the iron protocol and *HFE* gene analyses as part of the diagnostic process. Patients with hyperferritinemia and a LII-MRI ≥2 have significantly larger iron stores. This was based on the finding that patients with LII-MRI ≥2 had to mobilize a significantly higher amount of iron to reach iron depletion while patients with LII-MRI <2 had a significantly higher prevalence of components of the metabolic syndrome and had to mobilize a significantly lower amount of iron to reach iron depletion. It was concluded that LII-MRI is an effective method to help differentiating between major

and minor iron overload in patients being analyzed for hyperferritinemia. The LII-MRI it is not only suitable to find patients with *HFE*-related hemochromatosis but also to detect major iron overload caused by non-*HFE* hemochromatosis or secondary causes.

Part 2 - Current clinical aspects of HFE-related hemochromatosis and iron homeostasis

Although the prevalence of *p.Cys282Tyr* homozygosity is high, its phenotypic expression is low. In some patients there is no iron overload and depletion therapy is not indicated. In **chapter 4**, an overview of the population based *HFE*-related hemochromatosis South Limburg cohort is given. Data from all subjects with identified *p.Cys282Tyr* hemochromatosis in this geographical area enclosed by Belgium and Germany, with low migration rates, were collected. The aim of the study was to get a better insight in the epidemiology, phenotypic expression, disease manifestations and complications of *HFE*-related hemochromatosis and to create awareness to achieve an early diagnosis to prevent irreversible organ damage. The cohort contains 360 patients, followed for a median period of 9.9 years after their diagnosis. Remarkably fewer organ involvement and symptomatology were reported compared to what was previously published, possibly due to active iron depletion therapy and early diagnosis. The prevalence of hepatocellular carcinomas (HCCs) in our hemochromatosis population was reported and remarkably only 20 patients with a HCC were found of which 14 were found in a non-cirrhotic liver which is in contrast with previous publications were HCCs are almost exclusively found in cirrhotic livers.

The treatment of *HFE*-related hemochromatosis consists of two phases. The first one is the depletion phase in which the excess iron is removed while in the maintenance phase the re-accumulation of iron is prevented. The most frequently used treatment modality is bloodletting (phlebotomies). During each session 500ml of blood is removed, containing approximately 250 mg of iron. This is comparable with the amount taken for blood donation. In the iron depletion phase, phlebotomies are performed weekly until serum ferritin levels are below 100 µg/L or below 50 µg/L in case the transferrin saturation levels is >70%. The number of phlebotomies needed in the depletion phase is quite variable and depends on the amount of iron accumulated in the body. The number of phlebotomies in the maintenance phase varies between two and six per year. An alternative treatment is erythrocytapheresis, in which more erythrocytes can be removed per procedure and valuable blood components such as platelets and clotting factors can be returned to the patients. With this technique it is possible to remove more erythrocytes and thus iron per single procedure, nevertheless it is used less frequently due to costs and is only performed in specialized

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centers. However, in patients with severe iron overload this is a good alternative treatment.

Because of the variability in phenotypic expression it is difficult to identify patients in need for maintenance treatment, and when identified, how frequently treatment sessions should be applied. In **chapter 5**, retrospectively analyses of *HFE*-related hemochromatosis patients in the maintenance phase were investigated to research how to predict the patient's phenotype and thereby individualize treatment. The modified iron availability index (mIAI) was developed, calculated by serum ferritin levels at diagnosis divided by age at diagnosis minus 20 when male, and ferritin at diagnosis divided by age at diagnosis plus 20 when female. The mIAI seems a fairly good predictor in *HFE*-related hemochromatosis patients not taking proton pump inhibitors (PPIs) to differentiate patients needing ≥3 maintenance phlebotomies per year. Therefore, this index might help to select patients who benefit from an alternative less frequent therapy such as erythrocytapheresis.

Patients using PPIs were excluded since PPIs are known to have a significant effect on the amount of phlebotomies needed per year by lowering the amount of iron absorbed in the digestive tract. It is remarkable that in patients without *HFE*-related hemochromatosis iron deficiency is not a frequent side effect of chronic PPI use. In **chapter 6**, a proof of concept study was conducted in which serum iron and hepcidin levels after a pharmacological dose of 50 mg iron (Fe^{3+}) polymaltose were measured in patients with *HFE*-related hemochromatosis and in healthy controls. These measurements were repeated after seven days' treatment with PPIs. With this study, the reduction in iron absorption in *HFE*-related hemochromatosis patients in contrast to healthy control subjects was confirmed after lowering gastric acidity by PPIs. The assumption that a decrease in hepcidin concentration in healthy control subjects, in response to the reduced availability of Fe^{2+} for absorption, can explain the difference in iron absorption between these groups, could not be confirmed probably due to a small sample size.

HFE-related hemochromatosis is characterized by low hepcidin levels leading to continuous iron absorption and thus elevated serum ferritin and serum iron levels. In **chapter 7**, the case of a patient with *HFE*-related hemochromatosis with elevated hepcidin and normal iron levels during an episode of systemic inflammation was presented. When iron parameters and hepcidin levels were measured one year after his full recovery low hepcidin levels and higher iron levels were found as expected in a patient with *HFE*-related hemochromatosis. It was suggested that in the absence of a

proper functioning HFE, resulting in blockage of the BMP/SMAD pathway, the innate low hepcidin concentration can be upregulated by inflammation, probably via the JAK/STAT3 pathway. This is a rather unexplored area within *HFE*-related hemochromatosis research and could lead to new treatment possibilities.

Chapter 8 focused on hereditary aceruloplasminemia, a rare genetic condition in which iron gradually accumulates in the brain and various internal organs. This can lead to diabetes, retinal degradation and neurological disorders. It can also result in anemia since iron sequesters in the cells and is not available for the production of erythrocytes. The current treatment strategies are associated with many side effects as for example an aggravation of the already present anemia. Two hereditary aceruloplasminemia patients were treated with erythrocytapheresis in order to prevent iron re-accumulation after peripheral iron normalization by chelators. Erythrocytapheresis seems a good treatment possibility because it allows a more precise and selective reduction in erythrocytes preventing the development of symptomatic anemia. This is the first report on therapeutic erythrocytapheresis in hereditary aceruloplasminemia, that prevented progression of cerebral and peripheral iron accumulation without causing symptomatic anemia.

Finally, in **chapter 9**, an overview of the main findings is given and discussed. The thesis ends with a discussion on new insights and future perspectives in the field of interpreting hyperferritinemia and *HFE*-related hemochromatosis.