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TOPIC HIGHLIGHT

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Pathology of hepatic iron overload

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Abstract

Although progress in imaging and genetics allow for a noninvasive diagnosis of most cases of genetic iron overload, liver pathology remains often useful (1) to assess prognosis by grading fibrosis and seeking for associated lesions and (2) to guide the etiological diagnosis, especially when no molecular marker is available. Then, the type of liver siderosis (parenchymal, mesenchymal or mixed) and its distribution throughout the lobule and the liver are useful means for suggesting its etiology: HLA-linked hemochromatosis gene (HFE) hemochromatosis or other rare genetic hemochromatosis, nonhemochromatotic genetic iron overload (ferroportin disease, aceruloplasminemia), or iron overload secondary to excessive iron supply, inflammatory syndrome, noncirrhotic chronic liver diseases including dysmetabolic iron overload syndrome, cirrhosis, and blood disorders.

Key words: Iron; Liver; Biopsy; Hemochromatosis; Ferroportin; HLA-linked hemochromatosis gene; Hepcidin; Metabolic syndrome

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INTRODUCTION

Progress in molecular genetics and in liver imaging have allowed for the noninvasive diagnosis of most cases of inherited disorders of iron metabolism. However, liver pathology remains often useful to assess associated lesions-especially fibrosis-in patients with HLA linked hemochromatosis gene (HFE) and, mainly, nonHFE hemochromatosis, and to guide the etiological diagnosis of iron overload in the absence of a molecular marker. Moreover, because iron is more and more considered as a putative (co) factor of morbidity in patients with chronic liver diseases of various causes, iron excess must be recognized, indicated, qualified, quantified and interpretated even when it seems to be contingent upon a well established hepatic disorder.

ASSERTION OF IRON OVERLOAD

In the normal liver, iron is present at a concentration lower than 20 μmol/g of dry weight[1]. But, it is not histologically visible. Iron deposits are usually difficult to identify on usual stains unless they are abundant. Therefore, every liver biopsy should be routinely stained using not only hematoxylin-eosin-safron and connective stains, but also iron stain. Because it is easier and more reproducible than the Tirmann-Schmeltzer's method, Perls'staining is the most widely used, despite, it identifies Fe^{2+} only[2].

DESCRIPTION OF IRON OVERLOAD[2,3]

Distribution

The cellular distribution of iron deposits within hepatocytes, sinusoidal and portal macrophages, sinusoidal and portal endothelial cells, and biliary cells must be precisely described according to *lobular areas* in order to differentiate the following types of liver siderosis.

Parenchymal iron overload: It is related to intestinal iron hyperabsorption. Then, because it comes to the liver through the portal vein, iron deposits within hepatocytes as fine granules at the biliary pole of cells, and is distributed throughout the lobule according to a decreasing gradient from periportal to centrolobular areas. Mesenchymal iron deposits may be found at a latter stage when the amount of hepatocytic iron is high and responsible for sideronecrosis.

Mesenchymal iron overload: It corresponds to iron deposition within Kupffer cells and/or portal macrophages. Iron loaded cells are either isolated or grouped together without any lobular systematization. When associated, hepatocytic iron deposits are rough, sparse and usually located within cells close to iron loaded macrophages.

Mixed iron overload: It presents with the histological characteristics of the previous two types and corresponds usually to complex conditions or to massive iron loading.

Table 1 Histological grading of iron storage[2]

Grade	Ease of observation and magnification required
0	Granules absent or barely discernible at × 400
1	Granules barely discernible at × 250 and easily confirmed at × 250
2	Discrete granules resolved at × 100
3	Discrete granules resolved at × 25
4	Masses visible at × 10, or naked eye

Table 2 Histological grades of iron storage from Deugnier Y and Turlin B[3-12]

Hepatocytic iron	0, 3, 6, 9 or 12 According granules size In each Rappaport area	HIS 0-36
Sinusoidal iron	0, 1, 2, 3 or 4 According granules size In each Rappaport area	SIS 0-12
Portal iron	0, 1, 2, 3 or 4 According to % of iron overloaded macrophages, biliary cells, and vascular walls	PIS 0-12
Total iron score		0-60

HIS: hepatocytic iron score; SIS: sinusoidal iron score; PIS: portal iron score.

It is also important to precise whether iron distribution throughout the liver is homogeneous or not, i.e. whether lobules (or, in case of cirrhosis, nodules) are equally iron loaded or not[4,5].

Quantification

Biochemical hepatic iron concentration: Irrespective of the method used (colorimetry or atomic absorption), the biochemical determination of hepatic iron concentration (HIC) is considered as the reference method for quantifying iron in the liver[1,6] In normal subjects, HIC ranges from 10 to 36 μmol/g of dry weight. Iron excess is considered as mild up to 150, moderate between 150 and 300, and important above 300. Cases with HIC greater than 1000 are exceptionnal. Results obtained from fresh tissue and from deparaffinized blocks are equivalent[7]. Therefore, determination of HIC on deparaffinized tissue should be the rule because it allows for histological control. This is especially relevant when iron distribution is heterogeneous as in the cirrhotic liver[4,5].

Histological semiquantitative assessment: Several scoring systems have been proposed. The Scheuer's scoring system, either in its original presentation[8] or modified according to Rowe *et al*[9] or to Searle *et al*[2] is widely used because it is simple (Table 1). However, it was not satisfactorily validated. The system proposed by the authors (Table 2) was well validated in both hemochromatotic[10,11] and nonhemochromatotic iron overload disorders[12]. But, it remains mainly used for research purposes due to its relative complexity.

ETIOLOGICAL DIAGNOSIS

Both the type of iron overload and associated hepatic lesions may guide towards the right etiology (Table 3).

Table 3 Main causes of hepatic iron overload according to the histological type of siderosis and associated lesions

- Parenchymal iron overload
  - With normal liver
    - Early genetic hemochromatosis
    - Nontransfused dysmyelopoiesis
    - Hereditary aceruloplasminemia
  - With cirrhosis
    - Iron overload secondary to cirrhosis
- Mixed iron overload
  - With normal liver
    - Insulin resistance syndrome
    - Ferroportin disease
    - Transfused dysmyelopoiesis
    - Oral or parenteral iron supplementation
  - With steatosis or steatohepatitis
    - Insulin resistance syndrome
    - Chronic alcoholism
  - With chronic hepatitis
    - Hepatitis C or B
    - Wilson's disease
  - With intrahepatocytic inclusions
    - Porphyria cutanea tarda
  - With cirrhosis
    - Late genetic hemochromatosis
- Mesenchymal iron overload
  - With normal liver
    - Inflammatory syndrome
    - Repeated transfusions

Genetic iron overload

Genetic hemochromatosis: Genetic hemochromatosis[3] corresponds to 4 disorders transmitted as autosomal recessive traits, two with late onset (adult type: HFE hemochromatosis and iron overload related to mutation on the receptor transferrin 2 gene) and two with early onset (juvenile type related to mutations on the hemojuvelin or the hepcidin gene). From a histological point of view, these disorders are very similar because of a common pathophysiology consisting in an impairment of hepcidin production whose degree modulates the severity of iron burden[13].

In early GH, iron remains located within hepatocytes, at the biliary pole of cells. It is distributed according to a decreasing gradient from periportal to centrolobular areas. This results in a typical parenchymal iron overload pattern.

With the passage of time, hepatocytic iron load increases, and then periportal sideronecrosis occurs. Sideronecrosis is responsible for macrophage activation, which leads to both development of fibrosis, and redistribution of iron towards nonparenchymal cells. In the absence of other cause of chronic liver disease, cirrhosis develops when hepatic iron concentration exceeds 400 μmol/g. GH related cirrhosis consists of large fibrous septa resembling biliary cirrhosis, while preserving the vascular architecture of the liver for a long time. This likely explains why portal hypertension and hepatic failure are rare features in GH patients. According to series, 25% to 50% of GH patients are still diagnosed at the cirrhotic stage.

Liver cancer is a frequent complication in GH[10]. Most cases are hepatocellular carcinomas (HCC) developed in

a cirrhotic liver. However, some cases of HCC have been reported in GH patients with no cirrhosis, and frequency of cholangiocarcinoma reports is increasing[14]. Two types of preneoplastic lesions have been reported and should be sought for at histological examination: (1) Iron-free-foci[15] consist of sublobular nodular clusters of hepatocytes devoid of iron or with a low iron content within an otherwise iron-overloaded liver. Most often, they exhibit a proliferative pattern with either large or small cell dysplasia in 50% of cases. More than half the patients with IFF on their initial liver biopsy will develop. (2) Von Meyenburg complexes have also been reported as abnormally numerous in the surrounding liver of patients with GH complicated with cholangiocarcinoma[14].

Nonhemochromatotic genetic iron overload

The ferroportin disease: The ferroportin disease[16] is a dominant hereditary iron overload disorder characterized by phenotypic variability. In most cases, iron deposits are found within macrophages (mesenchymal type) with no significant fibrosis. This corresponds to the classical asymptomatic form with elevated hyperferritinemia contrasting with normal or mildly increased transferrin saturation. Rarely, iron is predominantly located within parenchymal cells, and the histological picture is similar to that of genetic hemochromatosis with, in some cases, either severe fibrosis or cirrhosis. Then, transferrin saturation is usually markedly elevated.

Hereditary aceruloplasminemia: Hereditary aceruloplasminemia[17,18] is an exceptionnal disease transmitted as a recessive trait. Under the lens, iron is found predominantly in parenchymal cells. No case of liver cirrhosis has been described even in the most iron overloaded cases.

African iron overload : African iron overload[19] is a rare disorder characterized by a mixed hepatic iron overload frequently complicated with cirrhosis. It is related to excessive iron intake, and likely underlaid by non*HFE* genetic factors.

Excessive iron supply

When brought parenterally: (i.e. through multiple transfusions), iron is initially localized within Kupffer cells and portal macrophages. With time, it is usually redistributed towards surrounding parenchymal cells, which results in a mixed and heterogenous pattern.

In case of excessive chronic iron intake: mixed hepatic iron overload may develop as reported in elite road cyclists[20].

Inflammatory syndrome

It is a frequent cause of mesenchymal hepatic siderosis related to a defect of iron release from Kupffer cells due to increased production of hepcidin[13]. Iron deposits are usually sparse and distributed throughout the lobule.

Noncirrhotic chronic liver diseases.

Dysmetabolic iron overload syndrome (DIOS): Dios is a frequent condition corresponding to the association of an unexplained hepatic iron overload with usually normal transferrin saturation and features of metabolic syndrome[21,22]. The histological pattern of DIOS is mixed, both mesenchymal (throughout the entire lobule) and parenchymal (predominating in periportal area)[23]. Iron excess is usually mild and averages 100 μmol/g. However, in 30% of cases, the hepatic iron concentration to age ratio exceeds 2, a threshold previously considered as highly suggestive of GH before the discovery of the *HFE* gene. Either steatosis or steatohepatitis is present in 50% of cases. Bridging fibrosis or cirrhosis is found in 12% of cases. Whether iron may be involved in the development of fibrosis in DIOS patients, and its removal may be beneficial remains debated.

Alcoholic liver disease: Mixed and mild hepatic siderosis is found in 5% to 20% of chronic alcoholics, even in the absence of cirrhosis. A direct effect of alcohol on hepcidin production could be involved[24].

Chronic hepatitis: Hepatic iron deposition is found in 35% to 56% of patients with chronic hepatitis. This was especially demonstrated in patients with chronic hepatitis C. The histological pattern is usually mesenchymal with frequent iron deposits in endothelial cells. Iron excess has been shown to be correlated with necrotico-inflammatory changes, and to decrease after interferon therapy. Moreover, iron removal before or at the time of interferon therapy could result in histological improvement, even in nonresponders[25].

Wilson disease: Mixed iron overload is frequently found in the liver of patients with Wilson disease. Its mechanism is likely multifactorial, and involves low serum ceruloplasmin levels, hemolysis, necrotico-inflammatory changes and cirrhosis.

Cirrhosis: Significant liver siderosis is found in 35 to 78% of patients with end-stage cirrhosis, irrespective of the cause of cirrhosis[4,5]. Iron deposits are mainly located within hepatocytes, predominate in remaining periportal areas, and, in some cases, can mimic GH. However, liver siderosis secondary to cirrhosis is distributed very heterogeneously from a nodule to another and absent from fibrous septa, biliary cells and vascular walls. This usually allows for correct diagnosis, and points to the need, in case of cirrhosis, for interpretating hepatic iron concentration according to histological findings. It is likely that nontransferrin bound iron (NTBI) plays a key role in the development of iron overload in cirrhosis[26]. Indeed, in severe cirrhosis, serum tranferrin levels are low due to hepatic failure which results in increased saturation of tranferrin and, then, in the appearance of NTBI, a special form of iron that is avidly taken up by hepatocytes.

Hepatocellular carcinoma: Parenchymal or mixed iron overload is frequently present in the nontumorous part of the liver of patients with hepatocellular carcinoma (HCC), whether they have cirrhosis or not[27]. This supports the

(co)carcinogenic role of iron suggested by numerous experimental and epidemiological studies[28].

Blood disorders

Porphyria cutanea tarda: Mixed, heterogeneous and mild hepatic siderosis is encountered in 60% to 70% of patients with porphyria cutanea tarda (PCT). Searching for intrahepatocytic inclusions when faced with mixed iron overload is suitable, because the clinical diagnosis of PCT may be missed.

Dyserythroipioiesis: In well compensated dyserythropoietic syndromes, intestinal iron absorption is increased secondary to impairment in iron incorporation into red cell precursors. With time, severe hepatic iron overload resembling GH may develop, even in the absence of blood transfusions. Once transfusions are required, iron deposits in both parenchymal and mesenchymal cells resulting in a mixed pattern.

PLACE OF LIVER BIOPSY IN THE MANAGEMENT OF IRON OVERLOAD DISORDERS

Recent progress in both imaging and genetics have resulted in reducing the role of liver biopsy in the diagnosis of hepatic iron overload.

Positive diagnosis: MRI allows for a specific and sensitive detection and reliable quantification of excessive hepatic iron content when comprised between twice the upper limit of normal and 300 μmol/g dry weight[29]. Then, liver biopsy is no longer necessary to ascertain iron overload.

Aetiological diagnosis: MRI also allows for classifying iron excess as parenchymal, mesenchymal or mixed according to organ iron deposition (liver and/or spleen and/or spine)[30]. Then it provides guidance for the aetiological diagnosis process, especially with respect to the first choice of genotyping tests. This was recently well illustrated by Pietrangelo *et al*[31] in patients with ferroportin mutations. Moreover, in these patients, MRI contributed to establish phenotypic/genotypic correlations, and to understand the pathophysiology of the disease by demonstrating, beside the classical mixed pattern of iron accumulation, a non-classical parenchymal pattern related to the N144H ferroportin mutation[31].

Disease severity: Once the positive and aetiological diagnosis of hepatic iron overload has been made, it is mandatory to assess the degree of hepatic damage, that is to determine whether severe fibrosis has developed or not. This will remain the major goal of liver biopsy as long as noninvasive tests for fibrosis-including biochemical markers, and elastometry-are not validated in patients with iron overload syndromes.

Currently, indication of liver biopsy can be discussed according to the phenotypic presentation of iron excess.

In case of hemochromatotic phenotype (= increased transferrin saturation with parenchymal iron deposition), performing liver biopsy depends on HFE genotyping:

In C282Y homozygotes, liver biopsy is no longer necessary for diagnosis, but remains suitable with respect to prognosis. Guyader *et al*[32] demonstrated that, when the liver was not clinically enlarged AND serum ferritin level was lower than 1000 ng/mL AND serum AST level was normal, there was never significant liver fibrosis (i.e. grade 3 or 4 fibrosis according to the METAVIR scoring system). On the contrary, when one, two or all these conditions were not met, there was a significant risk of fibrosis calculated as $1/(1 + \exp[-(-6.7620 + 3.2934\ AST_{(iu/l)} + 0.0013\ ferritin_{(ng/ml)} + 2.5317\ hepatomegaly_{(0:1)}])$. Accuracy of Guyader's algorithm was further validated in Canadian patients[32]. Since then, other equations of prediction of (non)fibrosis have been proposed[33,34] based upon age and serum ferritin level or serum ferritin level, serum AST level and platelets count. But, either they were not further correctly validated or they were not as simple for clinical use as Guyader's algorithm. Then, currently there is a global consensus to perform liver biopsy for fibrosis evaluation in C282Y homozygotes with either increased liver size, serum ferritin level higher than 1000 ng/mL or abnormal serum AST levels except when the diagnosis of cirrhosis is clinically obvious or when the predictive equation gives a risk close to 100%. Recently, Powell *et al*[35] showed that obesity-related steatosis may have a role as a cofactor in liver injury-especially fibrosis-in C282Y homozygotes. This has clinically important implications, but does not modify indications of liver biopsy in these patients.

In a C282Y-H63D compound heterozygote presenting with mild increase in transferrin saturation-usually comprised between 45% and 60% and in serum ferritin-usually < 500 ng/mL and with no biochemical abnormalities and clinical liver symptoms, it can be reasonably assumed that the HFE genotype is responsible for the abnormalities in iron metabolism[36], and that the patient is free of risk of fibrosis. Then, liver biopsy is not necessary.

In all other cases, the diagnostic procedure must be conducted irrespective of HFE genotype. Indeed, C282Y and H63D heterozygosity as well as H63D homozygosity not only are frequent (up to 1/3 of subjects in European general populations), but do not result, in a given subject, in clinically relevant perturbances of iron metabolism even if large genotyping studies[36-39] have shown that some of them induced slight but significant increase in serum ferritin and/or transferrin saturation. Then, liver biopsy remains suitable to search for an additional cause of either iron overload or chronic liver disease. The most frequent finding is heterogeneous parenchymal iron overload complicating alcoholic or viral liver cirrhosis[4,5,40]. Much more rarely, liver biopsy discovers marked iron overload suggesting an associated mutation on another gene involved in iron metabolism. In that case, the precise description of iron deposition, and associated lesions may participate in defining the choice of diagnostic molecular tests: mesenchymal or mixed iron deposition with no significant fibrosis is suggestive of ferroportin disease (which sometimes presents with TS > 60%) while parenchymal iron overload suggests the diagnostic

of juvenile haemochromatosis in a young adult with usually severe fibrosis (mutation on the hemojuvelin or hecidin gene) and that of transferrin receptor 2-related haemochromatosis in an adult with or without fibrosis.

In the absence of hemochromatotic phenotype (= low, normal or slightly elevated transferrin saturation), the question is whether increased serum ferritin levels are related to iron overload or not. MRI can replace liver biopsy to answer this question, and histological examination of the liver can be restricted to patients with significant iron deposition at MRI (i.e. hepatic iron concentration > 100 μmol/g dry weight) and/or elevated serum transaminase levels and/on abnormal noninvasive predictive tests of fibrosis[41]. In such a situation, the most frequent finding is mild and mixed iron overload with either metabolic or alcoholic steatohepatitis or chronic hepatitis C or porphyria cutanea tarda. Much more rarely, histological examination reveals marked iron overload with no significant fibrosis corresponding to ferroportin disease (mesenchymal type-normal or sligthly increased transferrin saturation) or to hereditary aceruloplasminemia (parenchymal type-low transferrin saturation).

Due to the widespread use of genotyping, of MRI and of noninvasive predictive markers of hepatic fibrosis, liver biopsy is less and less performed for diagnostic and prognostic purposes in C282Y homozygous patients. Conversely, it remains often necessary in other patients in order to guide the etiological diagnosis of hepatic iron overload by describing and semi-quantifying iron excess and by assessing associated lesions.

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