CURRENT TREND

Hepatic iron overload and risk of hepatocellular carcinoma in cirrhosis

Surcharge hépatique en fer et risque de carcinome hépatocellulaire sur cirrhose

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Summary  Iron accumulation in the liver is considered to be a co-factor for progression of liver disease. Iron overload can enhance the effects of oxidative stress and influence the natural history of patients with cirrhosis, exposing them to a higher risk of hepatocellular carcinoma. The results of clinical studies designed to assess the impact of liver iron content on the risk of tumor development have remained controversial for some time. It is known that common factors can affect both liver iron overload and the risk of cancer, necessitating multivariate analyses of these features in large cohorts of cirrhotic patients. Furthermore, the causes and consequences of hepatic iron overload appear to depend on the cause of the underlying liver disease. Thus, the only solid evidence of a relationship between liver iron overload and event occurrence has come from longitudinal studies conducted in homogeneous cohorts of patients with cirrhosis. So far, the available data suggest that iron accumulation in the liver is an independent risk factor for hepatocellular carcinoma in patients with alcoholic cirrhosis and/or nonalcoholic hepatosteatosis, but not in those with viral hepatitis C cirrhosis.

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Résumé  L’accumulation de fer hépatique semble être un co-facteur impliqué dans la progression des lésions hépatiques en accentuant les effets du stress oxydant et pourrait influencer l’histoire naturelle des malades ayant une cirrhose constituée et exposés au risque de survenue du carcinome hépatocellulaire. Les études cliniques ayant évalué son influence sur le risque de développer une tumeur ont été controversées. Les facteurs influençant cette surcharge et le risque de survenue du cancer étant communs, l’analyse de ces différents facteurs nécessitait la réalisation d’analyses multivariées complexes dans des cohortes de grande ampleur. Par ailleurs, les causes et les conséquences de cette surcharge semblent dépendantes de la cause de la maladie hépatique sous-jacente, la cirrhose et le carcinome hépatocellulaire représentant des stades évolutifs communs à des maladies de cause et de physiopathologie différentes.

Abbreviations: HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; TNF-α, tumor necrosis factor-α.

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Ainsi, seules les études longitudinales réalisées dans des cohortes de malades homogènes ont permis d’analyser les liens entre surcharge hépatique en fer et survenue d’événements, suggérant notamment que cette surcharge serait un facteur de risque indépendant de survenue du carcinome hépatocellulaire chez les malades ayant une cirrhose alcoolique et/ou liée au syndrome métabolique, mais non chez les malades ayant une cirrhose virale C.
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Introduction

Iron overload is considered to be a co-factor for the onset and progression of a large number of liver diseases, warranting a specific histopathology test [1]. The degree of iron overload depends on genetic, epidemiological and environmental factors, including the cause of the underlying liver disease.

There is a large body of evidence from human studies, and from animal and in vitro models, suggesting that liver iron content can affect tumor development via direct and indirect mechanisms [2]. The risk of HCC in patients with genetic hemochromatosis was recognized particularly in patients with overt cirrhosis [3]. The histological study of nontumoral liver samples obtained from rare cases of HCC developed in patients without cirrhosis has also suggested the role of iron in the development of liver cancer. In this setting, three case-control studies [4—6] have reported iron overload in tumor-free liver tissue in more than half of these patients and its association with a greater prevalence of heterozygous carriage of HFE gene mutations. This strong association between hepatic iron overload and HCC (irrespective of the cause of the overload and despite the retrospective nature of the reported series) suggests that iron probably plays a role in the genesis of liver cancer independently of other confounding factors commonly observed in cohorts of cirrhotic patients [7].

While the epidemiological data suggesting an association between liver iron and HCC are convincing, the controversy remains open as to the impact of iron overload on the risk of HCC in patients with chronic liver disease that has reached the stage of cirrhosis due to excess alcohol intake, NASH or viral infection. Most studies that have attempted to evaluate this link have been case-control studies, including patients with cirrhosis irrespective of the cause of the underlying liver disease. However, only longitudinal studies and/or studies conducted in well-defined cohorts with sufficiently prevalent events, thereby allowing multivariate analyses of all these parameters, can potentially resolve the question.

Determination of hepatic iron overload

In the normal liver, iron concentration is no greater than 20 μmol/g and iron deposits cannot be visualized on the standard histology slide. Histologists generally use Perls’ Prussian blue stain to reveal the ferrous iron (Fe^{2+}). Different types of iron overload can be distinguished by localizing the distribution of iron deposits to hepatocytes, sinusoidal spaces, macrophages and endothelial cells:

- parenchymal overload: linked to excess intestinal absorption, iron is deposited throughout the lobule via the portal system in a gradient that decreases from the periportal regions to the centrolobular regions;
- mesenchymal overload: macrophages and Kupffer cells are affected, with no lobular involvement;
- mixed overload: this can result from complex mechanisms or massive overload.

Iron chemistry is the gold standard for quantifying hepatic iron. Semi-quantitative histological methods have also been proposed, using more or less complex scoring systems [1]. The modified Deugnier score has been validated in several types of liver disease, and takes into account the different distributions related to cirrhosis-induced changes in liver architecture (Table 1) [8]. There are also noninvasive methods for determining liver iron content. Among the most recent, magnetic resonance imaging (MRI) has exhibited good correlation between hepatic iron concentration measured on T2-weighted images and iron chemistry performed on biopsy samples [9]. An algorithm has been developed, using the ratio between the liver and muscle signals, to determine hepatic iron overload from 60 to 375 μmol/g with good sensitivity and specificity (89 and 80%, respectively) irrespective of the underlying liver disease.

<table>
<thead>
<tr>
<th>Localization</th>
<th>Iron deposition in cirrhosis lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocytes</td>
<td>Perinodular Centronodular</td>
</tr>
<tr>
<td>Absent</td>
<td>0 0</td>
</tr>
<tr>
<td>Moderate</td>
<td>3 3</td>
</tr>
<tr>
<td>Non-confluent granules</td>
<td>6 6</td>
</tr>
<tr>
<td>Confluent granules</td>
<td>9 9</td>
</tr>
<tr>
<td>Sinusoidal cells</td>
<td>Perinodular Centronodular</td>
</tr>
<tr>
<td>Absent</td>
<td>0 0</td>
</tr>
<tr>
<td>Moderate</td>
<td>1 1</td>
</tr>
<tr>
<td>Non-confluent granules</td>
<td>2 2</td>
</tr>
<tr>
<td>Confluent granules</td>
<td>3 3</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Absent Focal Diffuse</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0 1 3</td>
</tr>
<tr>
<td>Biliary cells</td>
<td>0 1 3</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>0 1 3</td>
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</table>
and independently of the degree of fibrosis and, specifically, the presence of cirrhosis.

**Hepatic iron overload and chronic liver disease**

**Alcoholic liver disease**

Alcoholic liver disease is often associated with increased transferrin saturation, hepatic iron overload and elevated serum ferritin, which is poorly correlated with iron overload. Alcohol increases the intestinal absorption of iron [10] via decreased hepatic expression of hepcidin [11]. Oxidative stress induced by the metabolism of alcohol also triggers increased production of TNF-α in macrophages [12]. This cytokine, in turn, inhibits the synthesis of hepcidin [13]. Thus, alcohol has a direct impact on hepcidin via oxidative stress and an indirect impact via the production of TNF-α in Kupffer cells. In addition, in vitro and in vivo data both suggest that TNF-α also increases the expression of transferrin receptors on hepatocytes [14]. Such increased expression may also be induced by alcohol, leading to increased cellular iron uptake [15].

Iron deposits can be observed in hepatocytes and Kupffer cells of patients with alcoholic liver disease, especially in advanced stages [16]. Iron accumulation is positively correlated with the degree of cirrhosis [17]. The deleterious effects of alcohol and iron overload are synergistic, as has been widely observed in patients with hemochromatosis, as excess alcohol intake is associated with an increased risk of cirrhosis and cancer [18]. Nevertheless, mutations of the HFE gene in patients with alcoholic liver disease do not appear to be related to iron overload or disease severity [19—22]. Other genetic factors might affect iron overload in these patients, particularly genetic polymorphisms that modulate the response to mitochondrial oxidative stress. This would suggest a vicious circle involving these two parameters implicated in the progression of liver disease [23,24]. Thus, iron overload in the liver could be considered both a cause and a consequence of oxidative stress, and might be a useful prognostic marker of alcoholic liver disease.

**Chronic hepatitis C infection**

Moderate iron overload is sometimes observed in the liver of patients infected with the HCV. The overload is generally a mixed type and could be contributory to the progression of liver disease [25]. In addition, a number of studies suggest that the accumulation of iron or carriage of HFE mutations might have an impact on the response to antiviral treatment [26—28]. The pathogenetic mechanisms behind this accumulation are yet to be fully elucidated, but appear to involve viral infection via necroinflammatory and cytokine activity [29]. The viral genotype might also be involved, as iron overload may be greater with genotype 3 [30]. Reduced expression of hepcidin has also been described in HCV infection [31]. Although the data remain controversial, HFE gene mutations could be one of the host-related factor affecting iron accumulation and progression of hepatic fibrosis [26—28,32—38].

**Chronic hepatitis B infection**

Few studies have been devoted to the prevalence and significance of iron overload in hepatitis B virus (HBV) infection. In such cases, iron accumulation appears to predominate in hepatocytes [39]. Also, it may be linked to inflammatory activity and degree of fibrosis, but not to HFE gene mutation [25].

**Metabolic liver disease**

Hepatic iron overload is generally moderate and mixed (parenchymal and sinusoidal) in NASH [40], and might be contributory to the second ‘hit’ in the ‘two-hit’ theory proposed as an explanation of the wide spectrum of hepatic presentations [41]. In this case, iron overload could worsen oxidative stress in the liver and, thus, aggravate lipid peroxidation [42]. Moreover, the iron might have an effect on the development and aggravation of insulin resistance [43]. Indeed, iron overload and insulin resistance are associated in a unique nosological way [44—47], and may also be a condition predisposing to the progression of NASH [40,48—52]. The impact of HFE gene mutations in this case remains controversial [53—57], as it appears to vary with ethnic background and the cohort studied. Nevertheless, although there is no solid evidence that hepatic iron overload plays a role in the natural history of metabolic liver disease, iron depletion does appear to have a beneficial impact on indirect markers such as sensitivity to insulin and serum transaminase levels [58,59].

**Liver iron, HFE gene mutations and hepatocellular carcinoma**

While the degree of iron overload varies during the natural course of chronic liver disease, the development of cirrhosis is a common feature of progressive disease, warranting regular surveillance because of the risk of HCC [60]. If simple markers of high risk could be identified, specific preventative measures could then be implemented in selected populations. The impact of hepatic iron overload or HFE gene mutations on the risk of HCC in patients with cirrhosis has been the focus of a large body of research in recent years, yet the results have remained controversial because of their inconsistency, probably due to methodological biases (Table 2).

**Heterogeneous case-control studies**

Chapoutot et al. [61] compared hepatic iron content in 56 patients with HCC secondary to viral C cirrhosis versus 48 patients with viral C cirrhosis and no HCC. They found that HCC was associated with the presence of detectable iron deposits (odds ratio = 4.9) independent of age, gender or Child–Pugh score. Several researchers have reported that, after correcting for other risk factors such as age, gender and severity of cirrhosis, the prevalence of the HFE C282Y mutation was increased in patients with HCC [62,63]. Furthermore, the presence of
this mutation was also associated with hepatic iron overload.

However, other studies have failed to confirm these associations. Cauza et al. [64] studied patients with alcoholic and/or viral C cirrhosis and were unable to find an increased prevalence of HFE gene mutations in patients with HCC. Lauret et al. [22] found a discrete increase in the prevalence of these mutations solely in patients with alcoholic liver disease among a heterogeneous cohort. One French study, involving 133 consecutive patients with HCC secondary to cirrhosis, could find no increase in the prevalence of HFE mutations or in hepatic iron overload compared with a control group of 100 cirrhosis patients without HCC [65].

Prospective and/or homogeneous cohort studies

Selection bias might explain the inconsistent results of these studies. It is also accepted that hepatic iron content is affected by epidemiological and clinical factors (such as age, gender and disease severity) that are themselves linked with an increased risk of HCC in patients with cirrhosis. For this reason, cohort studies are needed to rule out such potential sources of bias and to enable multivariate analyses of the various confounding factors.

The first prospective study examining the impact of liver iron content on the prognosis of cirrhotic patients was conducted before the search for HFE gene mutations became a routine practice. The study included 229 consecutive patients with alcoholic cirrhosis (31%), viral C cirrhosis (53%) or a mixed form (16%) who were free of liver tumors at enrolment. These patients were followed regularly by HCC screening every 6 months [8]. Most of the patients had preserved their liver function (Child—Pugh class A: 72%; class B: 19%). During the follow-up period (57 ± 28 months), 95 patients died and 39 developed HCC. The modified Deugnier score was used to determine liver iron content and its effect on outcome (HCC or death). The initial liver biopsy was positive for iron deposition in 130 patients. However, there was no significant link between iron deposits in the liver at study entry and development of HCC during follow-up. Hepatic iron was nevertheless an independent factor predictive of death in a subgroup of patients with alcoholic cirrhosis, suggesting that iron deposition may have a deleterious effect on alcohol-induced, but not HCV-induced, histological lesions of the liver.

This first prospective study highlighted the importance of taking into consideration the cause of liver disease, and also the fact that liver failure and HCC are in competition as the leading risk factor for death. These findings might explain the inconsistency among the results of the earlier case-control studies. However, the small numbers of patients in the different subgroups in this study hampered analysis, suggesting that this type of study should be redesigned to include larger cohorts.

More recently, a prospective study of a large population of patients with cirrhosis (n = 301), divided into subgroups as a function of the underlying liver disease (alcohol, n = 162; HCV infection, n = 139; mixed causes excluded) enabled investigation of the link between hepatic iron overload (measured on histology) and the development of HCC [66]. In this study, liver iron content was higher in patients with alcoholic cirrhosis than in patients with viral C cirrhosis, despite their older age. In the viral C cirrhosis patients, iron overload was not associated with HFE gene mutations, and neither of the two factors affected the risk of HCC. Marked liver iron overload was frequent in patients with alcoholic cirrhosis (55% with an iron score > 0; mean iron score: 2.0 ± 3.0; mean follow-up: 66.1 ± 45.1 months; HCC during follow-up: 40 out of 162 patients) and was associated with the risk of HCC (HR, 4.1, range: 2.1—7.3; P < 0.0001). Iron overload was also associated with risk of death in this cohort, suggesting an underestimation of the carcinogenic impact of liver iron. This effect persisted in the multivariate analyses including

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Results for liver iron, HFE C282Y mutations and hepatocellular carcinoma (HCC) secondary to cirrhosis in the literature, mainly case-control studies including heterogeneous cohorts.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of study</td>
<td>Patients (n)</td>
</tr>
<tr>
<td>Chapoutot et al. [61]</td>
<td>Case-control</td>
</tr>
<tr>
<td>Hellerbrand et al. [62]</td>
<td>Case-control</td>
</tr>
<tr>
<td>Ganne-Carrie et al. [8]</td>
<td>Prospective</td>
</tr>
<tr>
<td>Fargion et al. [63]</td>
<td>Case-control</td>
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<tr>
<td>Lauret et al. [22]</td>
<td>Case-control</td>
</tr>
<tr>
<td>Cauza et al. [64]</td>
<td>Case-control</td>
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<tr>
<td>Boige et al. [65]</td>
<td>Case-control</td>
</tr>
<tr>
<td>Nahon et al. [66]</td>
<td>Prospective</td>
</tr>
<tr>
<td>Sorrentino et al. [67]</td>
<td>Case-control</td>
</tr>
</tbody>
</table>

ND: not determined; OH: alcoholic cirrhosis; HCV: viral hepatitis C cirrhosis; NASH: nonalcoholic steatohepatitis.
Table 3 Causes and consequences of liver iron overload in chronic liver disease.

<table>
<thead>
<tr>
<th>Cause of chronic liver disease</th>
<th>Proven or assumed cause of liver iron overload</th>
<th>Proven or assumed consequences of liver iron overload</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>Reduced synthesis of hepcidin by direct inhibition</td>
<td>Progression of hepatic lesions</td>
</tr>
<tr>
<td></td>
<td>Reduced synthesis of hepcidin by indirect inhibition via increased production of tumor necrosis factor-α</td>
<td>Hepatocellular carcinoma</td>
</tr>
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<td></td>
<td>Increased expression of transferrin receptors</td>
<td></td>
</tr>
<tr>
<td>Nonalcoholic steatohepatitis</td>
<td>Necrosis &amp; inflammation</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td></td>
<td>Virus-related factors</td>
<td>Progression of hepatic lesions</td>
</tr>
<tr>
<td></td>
<td>Reduced synthesis of hepcidin</td>
<td>Reduced response to antiviral treatment</td>
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<tr>
<td>Chronic viral hepatitis C</td>
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</tbody>
</table>

age, gender and severity of liver disease, and increased in proportion to the liver iron score. Furthermore, the prevalence of the HFE C282Y mutation was higher in patients with alcoholic cirrhosis and iron overload; the heterozygous state was associated with a higher risk of tumor development during follow-up.

Sorrentino et al. [67] recently reported higher liver iron overload in HCC patients in a cohort of 153 patients with NASH cirrhosis. Although this was not a prospective cohort, any potential bias was reduced by including a homogeneous population (NASH cirrhosis with no excess alcohol intake or viral infection) that was matched for epidemiological factors associated with the risk of HCC, but excluding patients with HFE gene mutations. Liver iron overload was mainly intrasinusoidal in the HCC patients, suggesting that, beyond liver iron content, the localization of the deposits might also have an impact on the potential for liver cancer.

**Conclusion**

The link between liver iron overload and the risk of developing HCC secondary to liver cirrhosis is now well established, especially in patients with alcoholic or metabolic liver disease (Table 3). In addition to the impact of the underlying liver disease, iron overload also depends on a complex set of polygenic epidemiological and genetic factors that are still, so far, poorly understood. Thus, in routine clinical practice, liver iron content should be determined to better identify those patients who have a higher risk of HCC and who might be expected to benefit from preventative measures. Iron depletion has demonstrated efficacy in reducing the rate of complications, improving survival and even bringing about the regression of liver lesions in patients with genetic hemochromatosis [68]. Indeed, multicenter randomized prospective trials need to be undertaken to assess the potential benefits of such iron depletion on the prognosis and prevention of HCC in patients with alcoholic and/or metabolic cirrhosis.

**Conflict of interest**

None.

**References**


Iron and hepatocellular carcinoma


