Liver iron is predictive of death in alcoholic cirrhosis: a multivariate study of 229 consecutive patients with alcoholic and/or hepatitis C virus cirrhosis: a prospective follow up study

N Ganne-Carrié, C Christidis, C Chastang, M Ziol, F Chapel, F Imbert-Bismut, J-C Trinchet, C Guettier, M Beauchand

Abstract

Background/Aims—A study was undertaken of liver biopsy samples from 229 consecutive patients with alcoholic or hepatitis C virus related cirrhosis who were prospectively followed until January 1996 to evaluate the influence of liver iron content on survival and the occurrence of hepatocellular carcinoma.

Methods—Hepatic iron content was measured with a validated semiquantitative score, and its predictive value for survival and the occurrence of hepatocellular carcinoma was assessed.

Results—130 patients had detectable iron at enrolment. During follow up (57 (28 months), 95 patients died and 39 patients developed hepatocellular carcinoma. No significant relation was found between hepatic iron and the occurrence of hepatocellular carcinoma. Conversely, the presence of iron was predictive of death in alcoholic patients (p = 0.007) by the log rank test but not in patients with hepatitis C virus related (p = 0.71) or mixed (p = 0.98) cirrhosis. The predictive value of hepatic iron content in patients with alcoholic cirrhosis was confirmed by the Cox model using either a binary coding (p = 0.009; relative risk = 2.27; 95% confidence interval 1.2 to 4.19) or the continuous values (p = 0.002).

Conclusions—These results suggest that hepatic iron enhances liver lesions caused by alcohol but not those caused by hepatitis C virus.

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Keywords: cirrhosis; liver; iron; survival; hepatocellular carcinoma; alcohol

Iron overload is very common in many types of non-biliary cirrhosis, and, in end stage liver disease, hepatic iron concentrations may reach the ranges of haemochromatosis. Haemochromatosis in these livers seems to be acquired and may be responsible in part for liver injury. Therefore, because of enhanced oxyradical production, even slightly increased amounts of iron may lead to liver injury, DNA alterations, and HCC in these patients.

The aim of this study was to investigate the influence of hepatic iron content on the occurrence of HCC and survival in patients with cirrhosis caused by alcohol, hepatitis C virus (HCV), or both. These conditions were selected because they are the main causes of cirrhosis in industrialised countries, they are often associated with increased liver iron content, and they may induce increased oxyradical production and lipoperoxidation in the liver.

Patients and methods

Patients

Between January 1987 and January 1993, 229 consecutive patients admitted to our hospital department with alcoholic and/or HCV related cirrhosis and without detectable HCC were included in an HCC screening study after giving informed consent. Cirrhosis was confirmed at enrolment by liver biopsy in all patients. HCV antibodies were detected in baseline sera (collected at enrolment and stored at −25°C) by a third generation enzyme linked immunosorbent assay (Ortho HCV 3.0). At enrolment, none of the patients were found to have HCC on the basis of an ultrasound examination and serum a fetoprotein levels below 50 ng/ml. Patients were prospectively followed until January 1996 (reference date of statistical analysis) or death. The population studied included 135 men and 94 women, mean (SD) age 60 (10) years. Causes of cirrhosis were alcohol (n = 71) (alcohol intake >80 g/day for more than 10 years), HCV (n = 121), and a combination of alcohol and HCV (n = 37).

Abbreviations used in this paper: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; ALT, alanine aminotransferase.
Patients with alcoholic or mixed cirrhosis had histological evidence of alcoholic hepatitis in 60% of cases (n = 47 in patients with alcoholic cirrhosis, n = 18 in patients with mixed cirrhosis). In patients with HCV related cirrhosis, the mean total Knodell score was 11 and the mean activity score assessed by the METAVIR index was 2.27. In these patients, genetic haemochromatosis was ruled out by the absence of a first degree family history of iron overload or liver disease, or of typical phenotypic symptoms of haemochromatosis such as arthralgias, skin pigmentation, and overt mellitus diabetes. None of the patients had venesection during follow up. Interferon treatment was begun in 89 patients with HCV antibodies (56.4%) at a mean dose of 3 MU three times a week for six months. Twenty seven patients (24%) had normal alanine aminotransferase (ALT) levels at the end of the treatment, and 19 (17%) had a sustained biochemical response. During the study, 42 of 108 alcohol abusers claimed to be abstinent (39%), and 33 patients claimed to have reduced their daily consumption to less than 30 g a day when questioned.

HCC SCREENING
The screening programme included clinical assessment, standard biochemical tests, serum α fetoprotein determinations, and ultrasonographic examination of the liver every six months. When a focal liver lesion or increased α fetoprotein levels were detected, tomodensitometry and, when possible, fine needle guided liver biopsy were performed.

HISTOLOGICAL IRON ASSESSMENT
Liver biopsy specimens were fixed in Bouin’s fluid and routinely processed. Sections 4 µm thick of each sample were stained with Perl’s iron stain. All slides were examined by two independent observers with no knowledge of the clinical data and outcome. To evaluate iron deposits, Deugnier’s histological hepatic iron score was adjusted for cirrhotic samples. As in Deugnier’s original score, iron content was evaluated in hepatocytes, sinusoidal cells, and connective tissue. However, instead of considering three zones in Rappaport’s acinus for hepatocytes and sinusoidal cells, the cirrhotic nodule was split into central and peripheral zones only (table 1). The second modification of the original score included taking into account the heterogeneity of iron deposits from one nodule to another. All nodules were individually analysed, and the global hepatocyte and sinusoidal cell score was the mean score of all cirrhotic nodules from one sample (range 0 to 33).

This cirrhosis adapted histological hepatic iron score was first validated in 22 liver samples from independent cirrhotic patients (15 men and seven women aged 68 (7) years) who underwent liver biopsy in January 1995, by biochemical measurement of hepatic iron (colorimetric determination as described by Barry and Sherlock). The biochemical measurements and histological scores of hepatic iron were well correlated in these 22 independent cirrhotic patients (r = 0.68, p = 0.0005).

STATISTICAL ANALYSIS
The mean end point was overall survival on the reference date of 1 January 1996. Patients who were living at the reference date were included, and patients who underwent liver transplantation because of terminal hepatic failure were considered dead on the day of transplantation and lost to follow up. Patients were counted on the date of their last consultation. The secondary end point was the time to occurrence of HCC. The statistical analysis used the failure time date methods. The Kaplan-Meier method was used to estimate death and the occurrence of HCC for each parameter noted at enrolment, and the distribution of the occurrence of death and HCC were compared with the log rank test. A significance level below 0.10 was used to select the variables in the Cox’s proportional hazards model using a stepwise backward procedure with a threshold of 0.05. For continuous parameters, two models were used: (a) the use of a binary coding by defining a threshold for each parameter; (b) the use of the continuous values. Relations between biochemical and histological hepatic iron content were studied by the Spearman test. Statistical analysis used the SAS package. All reported p values are two tailed.

Results
HEPATIC IRON CONTENT
The interobserver agreement reached 87% for histological assessment of hepatic iron content. Thirty six of 71 alcoholic patients (51%), 73 of 121 patients with HCV (60%), and 21 of 37 patients with cirrhosis related to both alcohol and HCV (57%) had stainable liver iron at enrolment. Seventeen patients had a liver iron score >10 (nine alcoholic, six HCV, and two mixed cirrhosis), and only three had liver iron in the range of haemochromatosis (score >20). These three patients (two men, mean age 68) had severe alcoholic cirrhosis (Child-Pugh B); two of them had histological lesions of alcoholic hepatitis; one had mixed cirrhosis with mild histological activity. None had stainable iron in biliary cells. Family studies and clinical features showed no evidence of haemochromatosis, but the Cys282Tyr mutation of the haemochromatosis gene, which had not been discovered at that time, was not searched for.
In patients with stainable liver iron, the mean liver iron score was higher in those with alcoholic (8.2, range 1–27) and mixed (7.1, range 1–24) cirrhosis than in those with HCV related cirrhosis who did not consume alcohol (5.5, range 1–14) (p = 0.001).

PATIENT CHARACTERISTICS, SURVIVAL, AND OCCURRENCE OF HCC

Table 2 shows the distribution of the baseline characteristics in patients with alcoholic, HCV related, or mixed cirrhosis, and indicates the predictive value of each variable for the occurrence of death. The mean (SD) duration of follow up was 57 (28) months (median 53 months, range 3–108). At the reference date (1 January 1996), 12 patients out of 229 were lost to follow up. Eighty nine patients had died and six patients who underwent liver transplantation because of terminal hepatic failure were considered dead on the day of transplantation. The cumulative incidence of death at five years was significantly higher in patients with alcoholic cirrhosis (50%) than in those with HCV related (27%) or mixed (30%) cirrhosis. The overall annual incidence of death was 8.7%. Cause of death was known in 86 patients. Death was due to liver disease in 75 (79%) patients: 52 had terminal hepatic failure and/or upper gastrointestinal haemorrhage, and 23 died from the complications of HCC. Extrahepatic causes of death were cancer (pancreas, n = 2; tongue, n = 1), heart disease or stroke (n = 5), severe lung infection (n = 1), suicide (n = 1), and terminal renal failure (n = 1). Thirty nine of 229 patients developed HCC. HCC was diagnosed on the basis of positive histological examination (n = 19), or a combination of a focal mass and progressively increasing serum α-fetoprotein levels above 500 ng/ml (n = 13), or a focal mass associated with characteristics of a highly arterially vascularised liver cell tumour which increased in size during follow up (n = 7).

HEPATIC IRON CONTENT AND SURVIVAL

Hepatic iron content at enrolment had predictive value for death in the whole population (p = 0.01 by the likelihood ratio test) when it was introduced continuously into a Cox model. As the severity of cirrhosis was appreciably different in patients with alcoholic cirrhosis and patients with HCV related cirrhosis (table 2), statistical analysis was performed according to the cause of cirrhosis. Using a binary coding, the presence of hepatic stainable iron at enrolment was predictive of death by the log rank test in patients with alcoholic cirrhosis when the threshold was either 1 (p = 0.007, fig 1A) or 5 (p = 0.04, fig 1B), but not in patients with HCV related (p = 0.71, fig 2A) or mixed (p = 0.98, fig 2B) cirrhosis. In multivariate analysis, the two models performed in patients with alcoholic cirrhosis showed that hepatic iron content at enrolment was an independent predictive factor of death. When a binary coding was applied, the predictive variables of death selected by the Cox’s model were Child-Pugh class B or C (p = 0.0002; relative risk = 3.57; 95% confidence interval (CI) 1.83 to 6.95), age > 50 years (p = 0.006; relative risk = 3.79; 95% CI 1.97 to 7.27), and the presence of stainable liver iron (p = 0.009; relative risk = 2.27; 95% CI = 1.23 to 4.19). Using the continuous values (for liver iron, age, serum albumin, and bilirubin), the predictive variables for death selected by the Cox’s model were: Child-Pugh class B or C (p = 0.0001), age > 50 years (p = 0.0001), presence of stainable liver iron (p = 0.002), and low serum albumin (p = 0.09). Stainable liver iron was still predictive of death in patients with alcoholic cirrhosis who allegedly stopped drinking during follow up (n = 42, data not shown).

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**Table 2** Baseline characteristics in patients with alcoholic cirrhosis and hepatitis C virus (HCV) related cirrhosis with or without alcohol intake, and predictive value of death (log rank test)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Alcoholic cirrhosis (n = 71)</th>
<th>HCV cirrhosis (n = 121)</th>
<th>Alcoholic + HCV cirrhosis (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Died p Value</td>
<td>Total Died p Value</td>
<td>Total Died p Value</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45 30 0.55</td>
<td>60 19 0.64</td>
<td>30 12 0.93</td>
</tr>
<tr>
<td>Female</td>
<td>26 15</td>
<td>61 16 0.82</td>
<td>7 3</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>14 5 0.02</td>
<td>10 10 0.18</td>
<td></td>
</tr>
<tr>
<td>≥50</td>
<td>57 40</td>
<td>109 32 0.002</td>
<td>27 13</td>
</tr>
<tr>
<td>Child-Pugh class</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>29 14 0.001</td>
<td>104 26 0.0004</td>
<td>30 10</td>
</tr>
<tr>
<td>B and C</td>
<td>42 31</td>
<td>15 9</td>
<td>7 5</td>
</tr>
<tr>
<td>Ascites</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No</td>
<td>35 18</td>
<td>104 27 0.003</td>
<td>31 11</td>
</tr>
<tr>
<td>Yes</td>
<td>36 27</td>
<td>16 8</td>
<td>6 4</td>
</tr>
<tr>
<td>Oesophageal varices</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None or small</td>
<td>39 20</td>
<td>117 32 0.04</td>
<td>30 11</td>
</tr>
<tr>
<td>Large (II/III)</td>
<td>32 25</td>
<td>4 3</td>
<td>7 4</td>
</tr>
<tr>
<td>Prothrombin activity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;70</td>
<td>52 32</td>
<td>34 16 0.0001</td>
<td>22 11</td>
</tr>
<tr>
<td>≥70</td>
<td>19 13</td>
<td>82 19 0.03</td>
<td>15 4</td>
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<tr>
<td>Bilirubin (µmol/l)</td>
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<td></td>
<td></td>
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<tr>
<td>&lt;17</td>
<td>21 10</td>
<td>64 15 0.03</td>
<td>16 5</td>
</tr>
<tr>
<td>≥17</td>
<td>50 35</td>
<td>50 19</td>
<td>21 10</td>
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<td>Albumin (g/l)</td>
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<td></td>
<td></td>
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<tr>
<td>&lt;38</td>
<td>58 39</td>
<td>49 24 0.001</td>
<td>27 11</td>
</tr>
<tr>
<td>≥38</td>
<td>13 6</td>
<td>68 11</td>
<td>10 4</td>
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<tr>
<td>Hepatic iron score</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;1</td>
<td>35 17</td>
<td>48 15 0.71</td>
<td>16 7</td>
</tr>
<tr>
<td>≥1</td>
<td>36 28</td>
<td>73 20</td>
<td>21 8</td>
</tr>
</tbody>
</table>
HEPATIC IRON CONTENT AND HCC

As HCC was the cause of death in 24% of all patients, we checked the predictive value of hepatic iron content for the occurrence of HCC. There was no statistically significant relation between hepatic iron content and the occurrence of HCC in the whole population (p = 0.09) and in the three aetiological groups of cirrhosis (alcohol, p = 0.26; HCV, p = 0.24; HCV and alcohol, p = 0.63). Nevertheless, there was a higher incidence of HCC in patients with stainable liver iron (cumulative incidence at five years: 21% v 11% in patients without any stainable iron). As the occurrence of HCC in our patients was low (n = 36), we did not perform multivariate analysis.

Discussion

This study used a semiquantitative histological hepatic iron score derived from that of Deugnier and colleagues10 and adjusted to cirrhotic livers. This score was validated in comparison with biochemical results in 22 independent patients with alcoholic cirrhosis. The correlation between our histological estimation and the biochemical determination of liver iron was good. Moreover, this histological iron score was very close to the recently published histological method of Deugnier for assessing liver iron overload.15 As there is a large topographic variation in the distribution of liver iron in cirrhosis, no method, even biochemical, is entirely reproducible or can provide an accurate measurement of overall liver iron content.16–17 Therefore a semiquantitative method based on a histological score was chosen for practicality. Hepatic siderosis was very common. As in recent reports, almost 60% of our patients had stainable iron, and iron overload may develop not only in alcoholic cirrhosis but also in HCV cirrhosis.11 Three patients with severe liver disease had a high liver iron content within the range of haemochromatosis. None had a first degree family history of iron overload or of liver disease, or phenotypic symptoms of haemochromatosis such as arthralgias, skin pigmentation, and overt mellitus diabetes, or stainable iron in the biliary cells, which is considered a hallmark of genetic haemochromatosis. As the study was performed before the mutation Cys282Tyr of the HFE gene had been identified, no data are available on the prevalence of this mutation in our patients. The mechanism by which liver iron overload develops in patients with cirrhosis and without genetic haemochromatosis is largely unknown. According to recent reports, the presence of a single copy of either of the two HFE mutations associated with genetic haemochromatosis does not influence liver iron content or the risk of fibrosis in alcoholic liver disease.18 Moreover, in chronic hepatitis C, the physiopathological influence of the Cys282Tyr mutation on iron overload must still be clarified. Hezode and colleagues19 have suggested that the Cys282Tyr mutation does not play a role in liver iron overload because its prevalence did not differ in patients with and without iron liver overload. Conversely, Pipperno and colleagues20 have suggested that, in patients with chronic hepatitis C, iron accumu-
lates in the liver as a result of an interplay between genetic and acquired factors, and alcohol intake certainly contributes. Moreover, the severity of cirrhosis should be taken into account.

The main result in this study is that patients with alcoholic cirrhosis and stainable liver iron had a lower survival rate than those without stainable iron, and that the risk of death correlated inversely with liver iron content. Although alcoholic and HCV related cirrhosis groups were not matched and the prevalence of severe liver disease was higher in the alcoholic group so that iron content may simply be a marker of worsening liver disease, multivariate analysis performed in each group showed that hepatic iron content was an independent predictive factor of death in patients with alcoholic cirrhosis and not in those with HCV related and mixed cirrhosis. The hepatic iron content in patients with HCV related cirrhosis was lower than in those with alcoholic cirrhosis. However, although in patients with HCV infection and high alcohol intake the mean liver iron content at enrolment was in the same range as in patients with alcoholic cirrhosis, it did not have any detectable effect on survival. The absence of any effect of liver iron overload on survival in patients with HCV infection with or without alcohol intake could be explained by a different pathophysiology of liver failure in viral and alcoholic induced liver diseases, with cirrhosis from mixed origin resembling HCV related cirrhosis. The relation between hepatic iron content and death in patients with alcoholic cirrhosis may be due to the deleterious influence of liver iron on the course of alcoholic liver disease. The discrepancy between the three groups of patients supports the physiopathological role of iron in the progression of alcoholic liver disease and argues against the role of liver iron as a non-specific marker of advanced cirrhosis. In our study, death was mainly due to liver failure and not to HCC. As none of the patients included had evidence of liver nodules or increased α-fetoprotein at enrolment, the incidence of HCC as a cause of death was low. Furthermore, despite the reported common presence of iron in the non-tumorous livers of patients with HCG, our study did not show any significant relation between hepatic liver content and the occurrence of HCC. However, this negative result must be considered with caution because of the limited number of events and a tendency towards a greater risk of HCC in patients with liver iron overload. Even if it were statistically significant, it would be difficult to attribute a pathophysiological role to liver iron in hepatocarcinogenesis without performing multivariate analysis and taking into account the well known factors in HCC such as sex, age, and severity of cirrhosis.

Oxidative stress and the increased formation of lipoperoxides play a key role in hepatic fibrogenesis and these processes may be enhanced by iron. It is well known that excessive alcohol intake is correlated with an earlier onset of hepatic fibrosis and reduced survival in patients with haemochromatosis.

Surprisingly, the influence of iron on the outcome of alcoholic liver disease have only recently been suggested. Hepatic iron content is usually lower in alcoholic cirrhosis than in genetic haemochromatosis. However, even if iron distribution among nodules is more heterogeneous and the ratio between Kupffer cells and hepatocyte iron overload is more in favour of Kupffer cells in alcoholic cirrhosis, there is a large overlap between the patterns of alcoholic cirrhosis and haemochromatosis and a differential diagnosis may be difficult for the pathologist. It is therefore tempting to suggest that liver iron overload can lead to liver failure in alcoholic cirrhosis as well as in haemochromatosis. Tsukamoto and colleagues have shown that oral iron intake significantly increases the incidence and severity of the fibrosis in a rat model of alcoholic liver disease. This result suggests that, in rats, hepatic iron may play a role in the worsening of alcoholic liver disease. Previously, Ferenci and colleagues showed that the long term administration of silymarin, a free radical scavenger, tended to increase survival in patients with alcoholic cirrhosis and not in those with non-alcoholic cirrhosis. This suggests that free radicals may play a role in the pathophysiology of alcoholic liver disease but not in chronic viral disease, which also supports our results. In conclusion, this study provides additional data to support the hypothesis of a synergistic effect between iron overload and alcohol in the worsening of alcoholic liver disease.

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