Liver iron excess in patients with hepatocellular carcinoma developed on viral C cirrhosis

C Chapoutot, M Esslimani, Z Joomaye, J Ramos, P Perney, C Laurent, P Fabbro-Peray, D Larrey, J Domergue, F Blanc

Abstract

Background—Liver iron deposits are frequent in viral C cirrhotic patients but their role is not well defined.

Aims—To investigate the effect of liver iron excess on the prevalence of hepatocellular carcinoma (HCC) in patients with viral C cirrhosis.

Methods—Hepatic iron was evaluated retrospectively using a semiquantitative method in liver biopsies of 104 viral C cirrhotic patients, 48 with HCC and 56 controls (HCC free). Corrected total iron score (0–60) was defined by the sum of three scores: hepatocytic iron score (0–36), sinusoidal iron score (0–12), and portal iron score (0–12), multiplied by 3/3, 2/3, or 1/3 according to the heterogeneous iron localisation in the nodules.

Results—After adjustment for known risk factors for HCC, regression analysis showed that iron deposits (corrected total iron score >0) were more frequent in HCC patients than in controls (odds ratio 4.94; 95% confidence interval 1.59–15.32; p=0.0056). The median of corrected total iron score was significantly higher in HCC patients than in controls (odds ratio 1.092; 95% confidence interval 1.01–1.13; p=0.021). This liver iron overload was sinusoidal (odds ratio 5.2; 95% confidence interval 1.82–15.11; p=0.0022).

Conclusions—Liver iron deposition was more frequent and more important in viral C cirrhotic patients with HCC than in HCC free controls. Liver iron overload seems to contribute to the development of HCC in patients with viral C cirrhosis.

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Keywords: liver; iron; viral C cirrhosis; hepatocellular carcinoma

Hepatitis C virus (HCV) infection is a major aetiological factor for hepatocellular carcinoma (HCC).1 But not all patients with HCV infection develop HCC and much effort has been directed towards determining high risk patients. In most cases cirrhosis or at least advanced hepatic fibrosis is found.2 Several additional risk factors for HCC have been identified in this population of viral C cirrhotic patients: male sex, increasing age, and chronic co-infection with hepatitis B virus.1,3,4 However, the mechanism by which HCV leads to HCC is not known. Moderate liver iron overload is frequent in HCV patients.6–9 Experimental studies support the hypothesis that iron may have a carcinogenic effect facilitating the development of HCC.10 In one recent case control study, it was suggested that hepatic iron overload may contribute to the development of HCC in cirrhotic or non-cirrhotic patients.11 These observations are not well documented in viral C cirrhotic patients.

In view of these considerations, we have examined the influence of liver iron overload on the prevalence of HCC in patients with viral C cirrhosis. Liver iron deposits were graded semiquantitatively in cirrhotic patients who had developed HCC and in controls without HCC.

Materials and methods

SUBJECTS

The study was performed in 104 patients with histologically proved cirrhosis who attended the Department of Internal Medicine, Surgery C and Department of Hepato-Gastroenterology, University Hospital of Montpellier, between January 1991 and April 1998. Histological diagnosis of cirrhosis was based on internationally accepted criteria.12 All patients had antibodies to HCV, as determined using a second or third generation enzyme linked immunosorbent assay. We reviewed retrospectively histological sections of nontumoral liver samples performed in two groups of patients. Forty eight patients had HCC confirmed by histological examination. The control group included 56 patients without HCC identified by a normal liver ultrasound and serum α fetoprotein level <20 ng/ml.

Exclusion criteria in the two groups were: liver sample less than 0.4 cm in length or multifragmented biopsies without portal septa; family history of haemochromatosis or classic clinical expression of the disease (skin pigmentation, cardiac failure, diabetes, hypogonadism, arthritis); haemolytic disease; porphyria cutanea tarda; body mass index >25; iterative blood transfusions; serological markers for hepatitis B virus infection: hepatitis B surface antigen, hepatitis B core antibody; antinuclear antibody titre >1:40; positive assays for antismooth muscle antibody; antimitochondrial antibody; abnormal ceruloplasmin; and α1 antitrypsin phenotype. In addition, no patient was infected with the human immunodeficiency virus.

The following data were collected: age; sex; duration of infection by HCV (when contami-
nation was very probable and a precise date; transfusion or drug addiction in the past year); alcohol intake, defined as consumption of more than 40 g of alcohol over more than 10 years; interferon alpha treatment; serum HCV RNA detected by nested reverse transcription-PCR; and oesophageal varices (small, grade I; large, grades II–III). The degree of impairment of liver function was estimated using the Child-Pugh score.

Results are expressed as mean (SD, range), or number (%). Comparisons between groups were performed using the Student’s t test for quantitative variables with Gaussian distribution and the Kruskal-Wallis test and Spearman’s test for those with non-Gaussian distribution. The χ² test or Fisher’s exact test was used for comparison of qualitative variables. Iron scores and predictive factors of HCC were subsequently included in a stepwise forward logistic regression analysis to take into account confounding factors. A p value <0.05 was considered statistically significant.

### Results

**Patient Characteristics**

Patient data are shown in table 1. HCC patients were older than controls (p=0.0004), were more often male (p=0.018), and had a history of chronic alcoholism more frequently (p=0.03). Data collection was incomplete for three parameters (duration of illness, serum HCV RNA, and oesophageal varices).

### Pathological Data

Liver samples were obtained by resection in 11 cases during surgical treatment of HCC. These biopsies were at least 1 cm from the HCC. In 93 cases the biopsies were performed before the diagnosis of HCC (median five years (range 1–10)) using a percutaneous method. Specimens were fixed in alcoholar Bouin’s liquid, embedded in paraffin, cut into 5 μm thick slices, and stained with haematoxylin–eosin, Masson’s trichrome, Sirius red, and Perls’ staining. Each biopsy was examined by the same liver pathologist (ME) with no previous knowledge of the corresponding group. Histological iron content was graded semiquantitatively according to Deugnier and colleagues. In the three areas of the Rappaport lobule, iron deposits were assessed using three different scores: hepatocytic iron score (HIS), 0–36; sinusoidal iron score (SIS), 0–12; and portal iron score (PIS), 0–12. The total iron score (TIS) = HIS + SIS + PIS. To quantify heterogeneous iron distribution, a coefficient of heterogeneity was calculated (screening at ×4 magnification) according to Turlin and colleagues. This coefficient of heterogeneity was 1/3 when iron distribution was very heterogeneous (iron deposits in 1/3 of the nodules), 2/3 when iron distribution was homogeneous (iron deposits in 1/3 to 2/3 of the nodules), and 3/3 when liver siderosis was homogeneously distributed (iron deposits in more than 2/3 of the nodules). Liver siderosis was finally quantified by the corrected total iron score: cTIS = TIS × coefficient of heterogeneity. Large liver cell dysplasia was screened for in all samples according to the morphological criteria proposed by Anthony and colleagues.

### Statistical Analysis

Results are expressed as mean (SD; range), median (range), or number (%). Comparisons between groups were performed using the Student’s t test for quantitative variables with non-Gaussian distribution and the Kruskal-Wallis test and Spearman’s test for those with non-Gaussian distribution. The χ² test or Fisher’s exact test was used for comparison of qualitative variables. Iron scores and predictive factors of HCC were subsequently included in a stepwise forward logistic regression analysis to take into account confounding factors. A p value <0.05 was considered statistically significant.

### Pathological Assessment of Iron

Liver samples were obtained by surgery in nine (18.7%) HCC patients and in two (3.6%) control patients (p=0.02); in the other cases they were obtained by percutaneous biopsy. Mean biopsy length was 11.8 (SD 7.1) mm in the HCC patients and 13.4 (SD 7.2) mm in controls (p=0.03). Univariate analysis revealed that iron deposits (cTIS >0) were found histologically in 41 (85.5%) HCC patients compared with 34 (60.7%) controls (p=0.005). cTIS, parenchymal (HIS), and mesenchymal (SIS, PIS) iron scores were higher in HCC patients than in controls (table 2). Localisation of sinusoidal iron deposits (SIS >0) was different between the two groups (table 3). Sinusoidal iron was exclusively at the periphery of nodules in 27.1% of HCC patients compared with 16% of controls, centronodular in 8.3% versus 21.5%, and diffuse in 37.5% versus 12.5% (p=0.002). In HCC patients, median cTIS values were comparable when the specimens were obtained by percutaneous biopsy or by surgery (p=0.65). Similarly, the length of samples obtained by percutaneous biopsy had no influence on cTIS in either group.

### Table 1 Characteristics of patients with HCC and controls

<table>
<thead>
<tr>
<th></th>
<th>Patients with HCC</th>
<th>Controls</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y) (mean (SD) [range])</td>
<td>66.1 (8.5) [46–83]</td>
<td>59.1 (10.5) [32–80]</td>
<td>0.0004</td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>40/8</td>
<td>35/21</td>
<td>0.018</td>
</tr>
<tr>
<td>Chronic alcoholism (%)</td>
<td>11 (22.9)</td>
<td>24 (42.8)</td>
<td>0.03</td>
</tr>
<tr>
<td>Duration of illness (y)</td>
<td>14.5 (5.5–37)</td>
<td>16 (10–35)</td>
<td>NS</td>
</tr>
<tr>
<td>Child-Pugh class (%)</td>
<td>A 26 (54.2)</td>
<td>A 38 (67.9)</td>
<td>NS</td>
</tr>
<tr>
<td>B 11 (22.9)</td>
<td>B 12 (21.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 11 (22.9)</td>
<td>C 6 (10.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV RNA+ (%)</td>
<td>32 (91.5)</td>
<td>52 (96.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Large oesophageal varices (%)</td>
<td>21 (56.7)</td>
<td>24 (48)</td>
<td>NS</td>
</tr>
<tr>
<td>Interferon treatment (%)</td>
<td>18 (37.5)</td>
<td>16 (32.1)</td>
<td>NS</td>
</tr>
</tbody>
</table>

### Table 2 Histological iron scores (median (range)) in HCC patients compared with controls

<table>
<thead>
<tr>
<th>Score</th>
<th>Patients with HCC (n=48)</th>
<th>Controls (n=56)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIS</td>
<td>6 (0–21)</td>
<td>0 (0–18)</td>
<td>0.036</td>
</tr>
<tr>
<td>SIS</td>
<td>1 (0–9)</td>
<td>0.5 (0–6)</td>
<td>0.0002</td>
</tr>
<tr>
<td>PIS</td>
<td>1 (0–9)</td>
<td>0 (0–6)</td>
<td>0.046</td>
</tr>
</tbody>
</table>

cTIS, corrected total iron score; HIS, hepatocytic iron score; SIS, sinusoidal iron score; PIS, portal iron score.
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nogenic influence. Liver iron could be a possi-
by class (0 or >0).

cTIS, corrected total iron score. In this model cTIS was entered
into the host DNA16 contrary to hepatitis B
RNA of HCV does not seem to be integrated
mechanism of carcinogenesis is not known.1

Major risk factor for HCC but the precise
Hepatitis C virus infection is recognised as a

PIS. In the three models, male sex and age were
while the two groups did not di-

5.2; 95% confidence interval 1.82–15.11),

HCC patients than in controls (p=0.002; OR

1.13). When the three scores were introduced
in HCC patients than in controls (p=0.021; odds ratio
(OR) 1.092; 95% confidence interval 1.01–
1.13). When the three scores were introduced
into the model, SIS was significantly higher in
HCC patients than in controls (p=0.0022; OR
5.2; 95% confidence interval 1.82–15.11),
while the two groups did not differ in HIS or
PIS. In the three models, male sex and age were
the other significant predictive risk factors of
HCC (table 4).

Eight parameters were introduced into the
logistic model according to a stepwise forward
procedure: age, sex, treatment by interferon,
consumption of alcohol, Child-Pugh class,
oesophageal varices, large liver cell dysplasia,
and cTIS. The analysis indicated that the
prevalence of iron deposits (cTIS >0) in patients
with HCC was significantly greater than that in controls (table 4). In a second
model, median cTIS was introduced with the
other variables. The cTIS was higher in HCC
patients than in controls (p=0.021; odds ratio
(OR) 1.092; 95% confidence interval 1.01–
1.13). When the three scores were introduced
into the model, SIS was significantly higher in
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HCC (table 4).

Discussion

Hepatitis C virus infection is recognised as a
major risk factor for HCC but the precise
mechanism of carcinogenesis is not known.1
RNA of HCV does not seem to be integrated
into the host DNA16 contrary to hepatitis B
virus DNA,17 which suggests an indirect carci-
nogenic influence. Liver iron could be a possi-
bble additional risk factor for HCC.10

Our study shows for the first time in a large
group of patients with viral C cirrhosis that
histological liver iron deposits were more
frequent and more important in patients who
had developed HCC compared with controls
without HCC. Multivariate analysis showed
that iron overload was correlated with HCC,
independent of the existence of other known
risk factors, and that iron excess was sinusoi-
dal. The two other parameters with independ-
ent predictive value for HCC were age and
male gender, as in previous studies.1–3 In a pre-
vious investigation, the hepatic iron index (liver
iron concentration/age) in 20 patients with
HCC developed on cirrhosis was significantly
higher than in controls (24 cirrhotic patients
without HCC),15 but the causes of cirrhosis
were not well described, in particular exposure
to hepatitis B or C viruses. In our series of
patients, all HCC risk factors were analysed to
determine precisely the role of iron. Co-

fection with hepatitis B virus has been

described as an additional aetiological factor
for HCC;1 hence these patients were excluded.
The effect of HCV genotype 1b has been scruti-
nised as another risk factor for HCC,18–19 but
three recent studies revealed no preferential
role of individual HCV genotypes.20–22 In our
series HCV genotyping was performed only in
32 patients and the influence of this variable
was not analysed.

A diagnosis of haemochromatosis in our
series cannot be strictly excluded because the
C282Y mutation was not assessed. But no
patient had a familial history of haemochroma-
tosis, clinical signs of the disease, or had
undergone venesection therapy. Moreover,
contrary to the liver pathology seen in genetic
haemochromatosis patients, iron excess was
mild or moderate with a heterogeneous distri-
bution, and was mainly located within mesen-
chymal cells.

Hepatic iron was estimated using a semi-
quantitative method rather than by direct
measurement. We believe that the method used
by Deugnier et al is the most reliable. There is
a good correlation between results obtained by
semi quantitative assessment and a quantitative
method.23 In contrast, Ludwig and colleagues23
reported that in cases of mild hepatic iron
abnormalities, iron quantitation may give false
negative results, particularly in a liver with
extensive fibrosis, but all patients in the current
study had cirrhosis. The semiquantitative
method also has the advantage that lobular and
cellular distribution of iron can be assessed.

Our findings do not contradict previous
studies showing that many patients with viral C
cirrhosis have liver iron overload. Ludwig and
colleagues24 found positive iron stains in 18 of
43 (41.9%) viral C cirrhotic patients using a
different grading method. In our series, chronic
alcohol use did not seem to have an effect on
iron overload, in common with Piperno and


colleagues25 prospective study in HCV patients
and in patients with haemochromatosis,15 but
in contrast with another report.26 We believe
that this difference may be explained by lack of
an accurate method of identifying and quanti-
fying precisely alcohol consumption.

The source of liver iron deposit is not clear.
Heterozygosity for one of the two mutations of
the HFE gene at position 282 (C282Y) or 63
(H63D) could play a role.9–10 But in other
groups, heterozygosity for the HFE gene
mutation had no influence on iron overload.26–28
A second hypothesis is that iron excess in the
reticuloendothelial cells could come from
damaged hepatocytes. Indeed, a significant
relationship was found between the severity of
histological activity and mesenchymal liver iron

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(cTIS, corrected total iron score. In this model cTIS was entered by class (0 or >0).)

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overload.26,27 Finally, Deugnier et al have suggested that sinusoidal iron excess could reflect the duration of the disease in patients with haemochromatosis.17 In the present study, we found no significant correlation between iron deposits (cTIS) and duration of illness. But this information was obtained in only 49 (47%) of 104 patients. Other studies are therefore necessary to clarify these hypotheses.

Whatever the source of sinusoidal iron (mutation of the HFE gene, reflection of disease duration, other causes), it seems to play a role in liver injuries. Experimental studies indicate that the cellular site of iron deposition in the liver is important for its carcinogenic potential. Dietary carbonyl iron used in an animal model to initiate the parenchymal deposition seen in early non-cirrhotic genetic haemochromatosis patients does not present any carcinogenic properties.28 Conversely, parenteral administration of iron dextran leads to reticuloendothelial cell iron deposition and has a direct role in hepatocarcinogenesis in the rat liver without cirrhosis.31 In our study, iron excess was mainly located within reticuloendothelial cells, and this is the main argument in favour of the role of iron deposits in hepatic carcinogenesis.

The mechanism by which increased liver iron may contribute directly to hepatocarcinogenesis still needs to be determined. One hypothesis is that iron could act by triggering the production of reactive oxygen species which may induce secondary lipid peroxidation, oxidative damage to proteins and nucleic acids, and finally initiate and promote carcinogenesis.26,32

In the present study, we found an association between mildly increased hepatic iron stores and HCC in viral C cirrhotic patients, which could represent a cause-effect relationship. These results may have important implications if they are confirmed by other studies. Liver biopsy should not only be practised to confirm a diagnosis of cirrhosis but also to assess hepatic iron deposits. The second implication is that phlebotomy therapy could be proposed in these patients to reduce the risk of HCC.

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