HCV liver disease in renal transplantation: a clinical and histological study

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Abstract. The prevalence of HCV infection is high in renal transplantation (RT) patients: 29% in our cohort of 399 RT recipients. The consequences of that infection on the liver have to be carefully assessed. Clinical chronic hepatitis was detected from ALT concentrations (> x 1.5 N) in only 26 patients (22%) with constant (15%) or fluctuating (85%) ALT elevation. Only three of 117 cases developed cirrhosis (3%). No liver cancer was noted. Liver biopsy was performed (mean interval = 60.2 months) in 62 patients with HCV infection alone. We found 26 cases (42%) of chronic active hepatitis (CAH) with a mean Knodell score as low as 6.1 (range: 3–12), a mean activity grade of 4.9, and a fibrosis stage of 1.3. Twelve patients (19%) presented with normal liver pathology and met the criteria of healthy HCV carriers (positive viraemia, normal ALT and normal liver). The rest presented with portal lesions, either inflammation or fibrosis. In addition, patient and graft survival rates did not differ in HCV+ recipients. To conclude, HCV infection did not appear too deleterious for the liver in this cohort of patients. There is therefore no contraindication for HCV-positive recipients to undergo renal transplantation.

Introduction

Hepatitis C virus (HCV) infection appears to be the major cause for chronic liver diseases in renal transplantation. According to studies [1–8], its mean prevalence is around 25% (range 8.2–55%). The contamination routes of that population have been described: previous chronic haemodialysis, blood transfusions during the dialysis period or as part of pre-transplantation protocols, the graft, or nosocomial transmission in exposed groups.

Authors have described the possibility of severe evolution to chronic liver disease such as the occurrence of sub-fulminating hepatitis, cirrhosis, liver carcinoma, or death directly attributable to the viral disease. That has not been noted by all authors and significant differences have been reported in the survival rate of patients and grafts between HCV-infected and HCV-non infected patients [9]. More frequent rejections have been reported [9]. Lastly, the indication for renal transplantation is questioned by certain authors in case of HCV infection of the recipient.

The aim of this investigation was to study HCV infection in a population of transplanted patients, from the histological standpoint in particular.

Materials and methods

Patients

Three hundred and ninety-nine patients, totalling 415 transplantations in the NDT Department were studied from August 1984 to December 1991. Immunosuppressive therapy was based on cyclosporin in association with azathioprine and corticoids. Patient follow-up began on the eve of transplantation, was continued throughout hospitalization, at 3 and 6 months after surgery, then at least once a year thereafter.

Techniques

The serum samples, selected from a serotheque, were tested with an ELISA II kit and a RIBA II confirmation kit. HCV RNA screening by Polymerase Chain Reaction technique (PCR) in sera was performed in a sub-group of patients who seroconverted (-/+) after transplantation. Transaminases (ALT) were monitored according to an identical time table with a significance threshold value equal to 1.5 times the normal values. HBV markers were also investigated, with delta virus if they were Hb surface antigen-positive.

A sub-group of 12 patients were subjected to viraemic quantization by PCR (Amplicor Roche®, to genotyping by PCR (InnoLIPA) and anti-HCV core IgM detection.

Liver biopsy specimen were examined with an optical microscope and in some patients the presence of HCV RNA was investigated by PCR on frozen samples. Liver biopsy puncture was performed either when ALT were elevated or systematically in HCV-positive recipients.
patients. In cases of chronic active hepatitis, Knodell's score was determined. An inflammation grade, including periportal and bridging necrosis (graded 0–10), hepatocyte necrosis and/or hepatocyte degenerescence (graded 0–4), portal inflammation (graded 0–4) was added to a fibrosis stage (rated 0–4). The global score was the sum of all the above scores (0–22).

The statistical tests used were the Chi-square test ($\chi^2$) with Yates correction, Student's t-test according to Statview® and the curves were computed according to Kaplan-Meier estimate with MacSurvival®.

Results

Prevalence of HCV infection

The mean age of the population studied was 41.7 years (range 8–65). There were 133 women (33.3%) and 266 men (66.6%). The mean follow-up duration was 75 months (range 24–145). One hundred and seventeen (117) patients out of 399 were infected by HCV (29.3%).

One hundred and three (25.8%) patients were HCV seropositive before transplantation and none of them lost their antibodies in the course of follow-up. Fourteen patients who had no antibodies at the time of transplantation and who later became seropositive were identified. In fact, systematic PCR screening for HCV RNA in serum before transplantation revealed that six out of 14 were in fact already HCV-infected. In the other eight patients, antibodies occurred after 12–17 months in three, and after 48–72 months in the other five.

With regard to the various proteins present in the kits that were used, positivity with the structural protein (genome core, c22-3) was the most prevalent ($P<0.00001$). Antibody specificities at the time of transplantation and at the end of follow-up were compared. Differences were significant for non-structural proteins: cl00-3 ($P<0.001$), 5-1-1 ($P<0.03$), c33-c ($P<0.02$); patients tended to lose antibodies directed against those proteins. There was no significant difference for antibodies directed against the genome core structural protein c22-3 ($P=NS$).

PCR detection of HCV RNA in serum was positive before transplantation in 93/103 patients (90.3%) and during the follow-up period in 106/117 cases (90.4%). In cases of serologically proven active chronic hepatitis, HCV RNA was always found in serum (100%) and in 15 of the 16 patients where it was investigated in the liver (93.7%). In cases of inflammation restricted to the portal area (portitis), HCV RNA was always present in serum (100%).

In cases where RIBA II revealed four positive bands, the prevalence of HCV RNA in serum was 96.9%. In cases where antibodies against proteins 33-c and c22-3 were found, the prevalence was 93.1%. When RIBA II results were issued as 'undetermined', i.e. only one of the four antibodies was detected and if it was directed against protein c22-3, prevalence of HCV RNA was 93%. The differences between these three results were not significant ($P=NS$). It became significant when correlating the prevalence of HCV RNA (38.5%) and other 'undetermined' results with the positivity of non-structural proteins.

The results on viruses B and D are not presented because they were not the subject of this communication.

Clinical liver affection

ALT was significantly more frequently increased in the population of HCV-infected than in HCV-negative patients ($P<0.001$). This ALT increase was found in 26 out of 117 (22.2%) HCV seropositive patients. It was fluctuating in 22/26 (85%) of those. In cases of histologically proven active chronic hepatitis, 9/26 (34%) of patients had ALT greater than the threshold and in 6/9 (66.6%) that level was fluctuating. When inflammation was restricted to the portal area (portitis), only one patient (10%) exhibited elevated and fluctuating ALT.

Liver pathology

Histology examination was performed in 62 patients. Only the results concerning HCV are reported below.

Twenty-six (42%) had chronic active hepatitis. Knodell's mean score was 6.14 (range 3–12) with a mean activity grade of 4.86 and a fibrosis stage of 1.26. The mean interval between transplantation and liver biopsy was 46.5 months (range: 1–93).

Three (4%) patients had cirrhosis (rated 4 on the fibrosis score) with 1, 48 and 63-month intervals between transplantation and liver biopsy.

Other histological results are reported in Table 1.

Healthy carriers

The 12-patients sub-group with normal liver was scrutinized. These were patients whose histological examination had not elicited any abnormality: one portal inflammation rated 0 to 1 and no fibrosis. Liver biopsy puncture was performed 60.8 months on average (range: 24–96) after transplantation.

These were eight men and four women, including four French and eight Italians whose mean age was 42.3 years (range: 16–65). Nine grafts originated from cadaver kidneys and three from live donors. All patients were under chronic haemodialysis for a mean

<table>
<thead>
<tr>
<th>Pathology</th>
<th>62 Liver biopsies</th>
<th>Mean interval (months)</th>
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<tbody>
<tr>
<td>HCA</td>
<td>26 (42%)</td>
<td>46.5 (1–93)</td>
</tr>
<tr>
<td>Portitis</td>
<td>10 (16%)</td>
<td>56.3 (24–76)</td>
</tr>
<tr>
<td>Normal</td>
<td>12 (20%)</td>
<td>60.8 (24–96)</td>
</tr>
<tr>
<td>Acute hepatitis</td>
<td>5 (8%)</td>
<td>42.2 (7–72)</td>
</tr>
<tr>
<td>Portal fibrosis</td>
<td>4 (6%)</td>
<td>41 (3–98)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (8%)</td>
<td>25.6 (7–60)</td>
</tr>
</tbody>
</table>
duration of 36.2 months (11–60) and all were preoperatively transfused. There was no extra-hepatic HCV affection. These patients were asymptomatic and had normal clinical examination. They were all HCV-infected at the time of transplantation, except one who became seropositive in the year following renal transplantation.

The mean follow-up duration was 71.9 months (36–108). Serum HCV RNA was found at each visit and ALT were always normal. Liver HVC RNA was found in 9/11 (82%) of the samples tested. Also, a quantitative study of viraemia revealed an average genomic copy count of $31 \times 10^4$ (1.6–67) per ml of serum. Genotype study revealed that seven patients were infected by genotype 1b, three were infected by genotype 2a, one by genotype 3a and one by genotype 4a. Anti-HCV IgM were detected in five patients who had a genome replication count of $41 \times 10^4$ per ml of serum (17–67) and four times genotype 1b and one genotype 3a.

All grafts were functional at the last follow-up visit. All patients were under cyclosporin treatment, 10 under azathioprine (45 mg/d on average) and nine were under prednisone (6.4 mg/d on average).

Renal transplantation results

Patient and/or graft survival and rejection incidence were not significantly different in the two populations (HCV- and HCV+).

Discussion

The prevalence of HCV infection in our population [2] was similar to that already described in the literature [6,10].

The strong correlation between positive RIBA II test and PCR detection of HCV RNA is consistent with persistent infection. The fact that no patient lost anti-HCV antibodies during follow-up also supports active infection. This has already been reported in a similar population. With regard to the 'undetermined' results with protein c22-3 alone, active infection was most often present. This was confirmed by other authors and was elucidated, at least in part, by third generation tests.

In a population of immunodepressed patients, who make few antibodies, the interest of identifying the viral genome has already been shown [1]. We were thus able to show that six patients were in fact infected before transplantation. The contamination route of patients who were previously free of any viral infection could be discussed as follows: antibody occurrence within 12–17 months following transplantation is consistent with peroperative contamination, whereas a 48-to 72-month interval would favour community transmission (nosocomial).

ALT elevation was significantly more frequent in HCV-infected than in HCV-negative patients. However, this study confirms that ALT is a poorly sensitive marker of C viral infection [6–10]. This has been clearly recognized in non immunosuppressed patients.

No deleterious effect of C viral infection was noted on the incidence of graft rejection and on patient and/or graft survival in both populations. Similar observations were made by other authors. Regarding rejection incidence, this finding clearly need to be put into perspective because multiple factors are to be taken into account: HLA histocompatibility, presence or absence of anti-lymphocyte antibodies, compliance to immunosuppressive therapy.

It has clearly been demonstrated that liver pathology is necessary to assess the consequences of HCV infection, regardless of the patients being immunodepressed or not [6,8]. We report a 42% incidence of chronic active hepatitis with low histological Knodell score. Moderate inflammation with marked fibrosis and silent evolution towards cirrhosis has been shown to be possible in immunodepressed patients. With regard to patients with cirrhosis, it is conceivable that it was already present at the time of transplantation in one patient assessed during the first post-transplantation month. It would have been of interest to this study to have kept a histological record of the HCV-infected patients before transplantation and to repeat liver biopsy 5 years later.

No case of liver failure or liver carcinoma was noted in our experience.

It is of particular interest to have isolated a subgroup of 12 patients who match the definition of HCV healthy carriers [3]: no liver lesions, normal ALT, no extra-hepatic affection known to date, circulating virus, even if we know that viraemia can fluctuate with time, and finally the virus was found in the liver of most patients. The series was too small to make any satisfying interpretation of the quantitative PCR study, or to establish a correlation with the level of anti-HCV IgM and genotype study. It has recently been suggested that tissue antigen HLA-DR5 may have a protecting effect by modulating immune response with minimum histological consequences [7].

Conclusion

HCV seropositivity, when discussing a possible renal transplantation, does not appear to be a contraindication. Liver pathology is required to assess the consequences of viral disease. Only patients with active cirrhosis should be refused transplantation. Patients with chronic active hepatitis and Knodell score > 5 justify treatment with interferon before transplantation, i.e. during the dialysis period. Lastly, HCV infection does not alter graft outcome.

References