Risk Factors for Recurrence of Hepatitis C After Liver Transplantation

J. Ignacio Herrero,* Andrés de la Peña,* Jorge Quiroga,* Bruno Sangro,* Nicolás García,* Iosu Sola,† Javier A. Cienfuegos,§ María P. Civeira,* and Jesús Prieto*

Recurrent hepatitis C is a frequent complication after liver transplantation for hepatitis C virus–related cirrhosis, but risk factors related to its development remain ill defined. Twenty-three patients receiving a primary liver graft for hepatitis C virus–related cirrhosis and with an assessable biopsy performed at least 6 months after liver transplantation were studied retrospectively. The end point of this study was to look for risk factors associated with the development of histologic hepatitis C in the graft. Thirty-six major variables were studied, and those reaching significance by univariate analysis were included in a multivariate analysis. Eighteen patients (78%) developed posttransplant hepatitis C. On univariate analysis, six variables showed significant predictive value: increased immunosuppression for treatment of acute rejection; pretransplant hepatocellular carcinoma; cumulative doses of prednisone at 3, 6, and 12 months after transplantation; and mean blood trough levels of cyclosporine in the first 6 months posttransplantation. On multivariate analysis, two variables retained independent statistical significance as predictors of hepatitis C recurrence, namely receipt of antirejection therapy ($P = .0087$) and lower mean cyclosporine levels in the first 6 months after transplantation ($P = .0134$). Therefore, recurrence of hepatitis C after liver transplantation seems to be at least partially related to posttransplantation immunosuppressive therapy.

Chronic hepatitis C virus (HCV) infection is present in 25% to 40% of liver-transplant recipients in Europe and North America.¹ In these patients, recurrence of hepatitis C is one of the most frequent causes of graft dysfunction, sometimes leading to graft failure or death.²⁴ Although persistence of HCV RNA in serum after liver transplantation (LT) is nearly universal,³ biochemical or histologic abnormalities due to recurrence of hepatitis C in the graft range between 41%⁶ and 100%⁷ in previous studies. Factors accounting for these variations remain ill defined.

In this study, we have assessed whether a number of major variables related to donor, recipient, and donor–recipient interaction constitute significant risk factors for the recurrence of hepatitis C after LT.

From the *Liver Unit, †Department of Pathology, and ‡Department of Surgery, Clínica Universitaria de Navarra, Pamplona, Spain.
Address reprint requests to J. Ignacio Herrero, Liver Unit, Clínica Universitaria de Navarra, Avda Pío XII S/N, 31008 Pamplona, Spain.
Patients and Methods Study Population

Between May 1990 and December 1994, 31 adult patients with HCV-related cirrhosis underwent a primary LT from a blood group–identical donor at our institution. Their age was 55 ± 1 years (mean ± SEM). Twenty-one of them were men, and 15 (48%) had irresectable hepatocellular carcinoma confined to the liver (one to three neoplastic nodules with a maximum diameter of 5 cm). All patients were anti-HCV–positive by a second-generation test and exhibited detectable HCV RNA in serum before LT. To be eligible for the study, the patients had to have an evaluable liver biopsy performed at least 6 months after LT. Eight patients were excluded: 3 refused liver biopsy, 1 had a diagnosis of recurrent hepatocellular carcinoma 6 months after LT, and 4 died before 6 months after LT. Causes of death of these patients were recurrent hepatocellular carcinoma (1 patient), bacterial pneumonia (2 patients), and disseminated fungal infection (1 patient). Three of the 8 patients excluded had been diagnosed of recurrent hepatitis C (cholestatic in 1 case), but they were excluded because they did not have a biopsy performed after the sixth postoperative month and hepatitis C was not the cause of death.

Immunosuppression

All patients received cyclosporine-based immunosuppression. Cyclosporine therapy was started on the second postoperative day by intravenous infusion until the patient was able to take oral medications. Doses of cyclosporine were adjusted to obtain the following trough blood levels (measured by monoclonal radioimmunoassay): 250 to 300 ng/mL within the first 3 months, 200 to 250 ng/mL from 4 to 6 months, and 175 to 225 ng/mL from this time until the end of the first year post-LT. Azathioprine was administered at a dose of 1 to 2 mg/kg/d from the immediately postoperative day. In cases of anemia, thrombocytopenia, or granulocytopenia, azathioprine dose was reduced or discontinued. Four patients were in a clinical trial using azathioprine only for the first month. All the patients received 1 g of methylprednisolone in the anhepatic phase of surgery. Decreasing doses of methylprednisolone were administered until the postoperative day 5. From postoperative day 6 until the end of the sixth month post-LT, prednisone was administered at a dose of 20 mg/d, and then at a dose of 15 mg/d until the end of year 1. Four patients received monoclonal antibodies in the immediate postoperative period: 2 patients received BT563 (BiostestPharma, Dreieich, Germany), a murine monoclonal antibody against CD25, as a part of a clinical trial, and 2 patients were given OKT3 (Ortho, Raritan, NJ) because of postoperative renal failure and severe cyclosporine toxicity, respectively.

Graft rejection was diagnosed during liver biopsy. Episodes of graft rejection were treated with three doses of 1 g of methylprednisolone bolus administered on 3 consecutive days followed by a 5-day progressive dose tapering. Steroid-resistant episodes were treated with OKT3 for 10 to 14 days.

Histologic Assessment and Definition of Hepatitis C

Liver biopsies were obtained 6 and 12 months after LT and yearly thereafter (at least in those patients who had not had recurrent hepatitis) according to a previously established clinical protocol and when clinically indicated at any time. Histologic samples from the native liver and all the post-LT biopsy specimens from these patients were retrospectively assessed by a single senior pathologist who was unaware of each
patient’s clinical condition and looking for histologic evidence of hepatitis.

The diagnosis of hepatitis C recurrence required the coexistence of histologic hepatitis and the detection of HCV RNA in serum. Other causes of hepatitis, mainly cytomegalovirus and hepatitis B virus, were ruled out by serologic testing and histopathologic findings, including in situ hybridization for cytomegalovirus and detection of hepatitis B surface antigen and hepatitis B core antigen. According to Henley et al., histologic diagnosis of hepatitis required the coexistence of parenchymal disorganization, with presence of necrotic hepatocytes, sinusoidal lymphocytosis, Kupffer cell activation, and portal lymphocytosis. All patients with recurrent hepatitis C had simultaneously abnormal results of liver function tests. The date of recurrence was defined as the postoperative day on which the abnormal results of liver function tests leading to liver biopsy were first detected.

**Analysis of Potential Risk Factors for Recurrent Hepatitis**

Thirty-six potential risk factors for hepatitis C recurrence were assessed:

- **Related to the recipient:** Age; sex; blood group; the most frequent HLA A, B, and DR antigens; pre-LT liver function tests; histologic activity index of the native liver according to Knodell et al.; and presence of hepatocellular carcinoma in the explanted liver.
- **Related to the donor:** Age, sex, and the most frequent HLA A, B, and DR antigens.
- **Related to donor–recipient interaction:** Sex and HLA A, B, or DR match.
- **Related to surgery:** Intraoperative requirements of red blood cells, fresh-frozen plasma, platelets, or cryoprecipitate, and intraoperative use of prostaglandin-1 and aprotinin.
- **Related to postoperative immunosuppression:** Three-, 6-, and 12-month cumulative doses of azathioprine and prednisone; 3-, 6-, and 12-month mean cyclosporine levels; use of monoclonal antibodies and use of increased immunosuppression for treating acute rejection episodes. Mean levels of cyclosporine were calculated obtaining the area under the curve formed by all the trough cyclosporine levels obtained in the period of study and dividing it by the number of days of this period.

**Statistical Analysis**

Hepatitis recurrence curves were generated using the Kaplan-Meier method. The Cox proportional hazards regression model was used to identify risk factors for hepatitis C recurrence. Initially, an univariate Cox analysis was performed for every potential risk factor. Those variables showing a *P* value of < .05 on univariate analysis were assessed on a multivariate Cox model. Relative risks and 95% confidence intervals for these relative risks were obtained.

**Results**

Eighteen (78%) of the 23 evaluable patients developed post-LT histologically proven hepatitis C recurrence after a median period of 73 days (range, 25 to 538 days) (Fig. 1). All patients diagnosed with hepatitis C had abnormal serum aminotransferase levels at the moment of liver biopsy. In no case did recurrent hepatitis C lead to graft failure or death. In most cases, recurrent hepatitis was mild or moderate in activity. Nineteen patients had evaluable liver biopsies 1 year after LT: 7 were normal, and in the rest,
histologic activity index was between 1 and 5 in 6 patients, between 6 and 10 in 3, and higher than 10 in 3 patients. In no case did recurrent hepatitis lead to graft failure or death, although 1 patient developed cirrhosis because of chronic HCV infection 3 years after LT. Currently, the patient remains compensated and with good graft function 5 years after LT. Follow-up of the patients with and without recurrence of hepatitis ranged from 534 to 2,149 and 577 to 1,758 days, respectively.

Six of the potential risk factors were significantly related to post-LT hepatitis C recurrence in the univariate analysis: 3-, 6-, and 12-month cumulative doses of prednisone, treatment of rejection, mean cyclosporine levels in the first 6 months after LT, with the presence of hepatocellular carcinoma (Table 1).

In the multivariate analysis, only two variables were independently associated to recurrence of hepatitis C. Those patients receiving treatment for acute graft rejection episodes developed more frequently recurrent hepatitis C (relative risk, 2.23; 95% confidence interval, 1.22 to 4.08, \( P = .008 \)). The association between treatment of acute rejection episodes and recurrence of hepatitis C is shown in Figure 2. This association was also chronologically evident, because all of the 8 patients who were treated for an episode of acute rejection developed recurrence of hepatitis C later, and in 7 (87%), recurrence occurred within the 6 weeks following antirejection treatment. There was a negative association between mean blood trough levels of cyclosporine and the recurrence of hepatitis C, meaning that the higher the mean cyclosporine levels were in the first 6 months after LT, the lower the hepatitis C recurrence risk was (the relative risk associated to the increase of 1 ng/mL in mean cyclosporine levels was 0.97; 95% confidence interval, 0.95 to 0.99; \( P = .01 \)). As shown in Figure 3, all patients with a mean cyclosporine level less than 250 ng/mL developed recurrent hepatitis.

Discussion

Despite the nearly universal persistence of hepatitis C viremia after LT for HCV-related cirrhosis,\(^5\) hepatitis C recurrence in the graft is not universal. Therefore, accelerated viral replication resulting from immunosuppression does not seem to be the only factor responsible for recurrence.\(^4,11,12\) In fact, high HCV RNA concentrations may be found in liver parenchyma of patients with an otherwise biochemically and histologically normal liver.\(^13,14\)

Feray et al\(^15\) showed a higher frequency of recurrent hepatitis in those patients infected with HCV 1b genotype, but this finding was not confirmed by other investigators.\(^16,17\) In the present series, the possible influence of HCV genotype on hepatitis C recurrence could not be investigated because 20 patients were infected by genotype 1b, 2 by genotype 1a, and 1 patient had a mixed 1a-1b infection.

Our findings confirm the relationship between recurrent hepatitis C and treatment of rejection episodes that has been previously reported.\(^2,18\) Furthermore, our results suggest a chronologic association between treatment of acute rejection and post-LT hepatitis C, as most patients treated for acute rejection developed hepatitis C within a few weeks. As previously suggested by Gane et al,\(^19\) this association may be due to the accelerated viral replication that follows severe suppression of the immune response induced by antirejection therapy.

The possible influence of baseline immunosuppression on the recurrence of hepatitis C remains unclear. The reported recurrence rate has been similar among patients receiving cyclosporine or tacrolimus as primary immunosuppressant,\(^2,20\) although Sheiner et al\(^5\)
observed that recurrence appeared earlier in patients treated with tacrolimus than in those receiving cyclosporine. Farges et al\(^2\) did not find any relation between immunosuppressive therapy and post-LT hepatitis C. In our series, cyclosporine seems to have a beneficial effect. Patients with the highest mean trough blood levels of cyclosporine in the first 6 months after LT had a significantly lower risk of hepatitis recurrence. This effect seems to be independent of the risk of rejection, as we did not find any relationship between cyclosporine levels and rejection. Although it is difficult to explain the possible link between a more intense immunosuppression and protection against hepatitis recurrence, it may be related to a downregulation of immune response against HCV, which could play a part in the pathogenesis of HCV-induced liver damage.\(^2\) This relationship between mean trough cyclosporine levels and hepatitis C must be investigated in larger series, as they do not reflect either the high variability of individual levels during the study period or the true quantity of immunosuppression, which is more closely related to the area under the curve. In conclusion, recurrence of hepatitis C after LT seems to be at least partially related to post-LT immunosuppressive therapy. Specifically, the close chronologic association between treatment of acute rejection episodes and hepatitis C recurrence in the graft strongly suggests a causal relationship. These data provide a rationale basis for administering antiviral therapy (a-interferon, ribavirin) to patients being treated for acute rejection. On the other hand, if the results reported here are confirmed in other series, a rapid tapering of cyclosporine might be avoided in patients transplanted for HCV-related liver cirrhosis.

References

Figure 1. Actuarial recurrence (Kaplan-Meier) of hepatitis C after LT. Number of patients at risk at 3, 6, 12, 18, and 24 months are indicated.

Figure 2. Comparison of actuarial hepatitis C recurrence (Kaplan-Meier) after LT according to the need for rejection treatment. Number of patients at risk at 3, 6, 12, 18, and 24 months are indicated.

Figure 3. Comparison of actuarial hepatitis C recurrence (Kaplan-Meier) after LT according to mean cyclosporine levels in the first 6 months. Number of patients at risk at 3, 6, 12, 18, and 24 months are indicated. (CyA, cyclosporine)
<table>
<thead>
<tr>
<th></th>
<th>3 Months</th>
<th>6 Months</th>
<th>12 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rejection treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 8)</td>
<td>75</td>
<td>87</td>
<td>100</td>
</tr>
<tr>
<td>No (n = 15)</td>
<td>33</td>
<td>53</td>
<td>60</td>
</tr>
<tr>
<td><strong>Hepatocellular carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 10)</td>
<td>30</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>No (n = 13)</td>
<td>61</td>
<td>77</td>
<td>77</td>
</tr>
<tr>
<td><strong>Prednisone 3 months (g)</strong>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.72-3.88 (n = 12)</td>
<td>25</td>
<td>50</td>
<td>58</td>
</tr>
<tr>
<td>3.89-16.43 (n = 10)</td>
<td>73</td>
<td>82</td>
<td>91</td>
</tr>
<tr>
<td><strong>Prednisone 6 months (g)</strong>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.86-5.76 (n = 11)</td>
<td>27</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>5.77-21.84 (n = 11)</td>
<td>73</td>
<td>82</td>
<td>91</td>
</tr>
<tr>
<td><strong>Prednisone 12 months (g)</strong>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.01-8.89 (n = 11)</td>
<td>27</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>8.90-25.44 (n = 10)</td>
<td>70</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td><strong>Cyclosporine levels (ng/mL)†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>215-275 (n = 10)</td>
<td>60</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>276-329 (n = 10)</td>
<td>40</td>
<td>40</td>
<td>60</td>
</tr>
</tbody>
</table>

*Cumulative doses of prednisone (in grams) during the first 3, 6, or 12 months after liver transplantation. †Mean blood trough levels of cyclosporine in the first 6 months after liver transplantation.