Old donors in liver transplantation for chronic hepatitis C

V. Aguilera, M. Ponce, M. Berenguer, R. Moreno1, J. M. Rayón2, F. Sanjuán3, M. Prieto and J. Mir1

Hepatogastroenterology Service, University Hospital La Fe, Ciberehd. 1Gastroenterology Service. Hospital de Especialidades LMN SXXI del IMSS. México D.F. 2Pathology Service. Surgery and Liver Transplantation Unit. University Hospital La Fe. Valencia, Spain

RESUMEN

Introducción: la historia natural de la hepatitis C recurrente tras el trasplante hepático (TH) es muy heterogénea, existiendo un porcentaje no despreciable de pacientes con evolución desfavorable. La identificación de factores asociados con peor evolución puede ayudar a mejorar el pronóstico de estos pacientes. La edad del donante se perfiló como uno de los factores más importantes, pero es una variable difícilmente modificable.

Objetivos: a) describir la historia natural de los receptores VHC (+) en función de la edad del donante (< o ≥ 50 años) incluyendo la evolución clínica, analítica e histológica; b) identificar en el grupo de receptores de donantes ≥ 50 años, aquellos factores preoclusivos asociados con una evolución agresiva.

Métodos: estudio descriptivo y retrospectivo de la evolución clínica e histológica mediante biopsias de protocolo, de 162 trasplantados VHC (+) entre 1997-2001 con tiempo de seguimiento post-trasplante de al menos 12 meses. La hepatitis C relevante del injerto se definió por la progresión a fibrosis mayor a 1 durante el primer año, desarrollo de hepatitis colestásica fibrosante y/o pérdida del injerto por enfermedad VHC recurrente en cualquier momento durante los primeros 5 años. Los factores analizados como posibles factores predictivos de hepatitis C relevante fueron: a) relacionados con el receptor: demográficos (edad, sexo), pre-trasplante (hepatocarcinoma, estado de Child, alcohol, marcadores serológicos del VHB, tratamiento antituberculosis, estado nutricional, analítica); b) relacionados con el donante: demográficos (edad, sexo), causa de fallecimiento, grado de estatosis definido como ausente o mínima vs. moderada-grave > 10%; c) relacionados con la cirugía: tiempo isquemia fría y recalentamiento, duración intervención, número de concentrados de hematíes transfundidos; y d) relacionados con el post-trasplante: inmunosupresión, analítica en el post-TH precoz (< 14 días), hepatitis aguda post-TH, complicaciones quirúrgicas precoz (vasculares y/o biliares).

Resultados: los pacientes se dividieron en dos grupos según la edad del donante (< 50 años = grupo 1, n = 83, 51% y ≥ 50 años = grupo 2, n = 79, 49%). La mediana de seguimiento fue de 5 años (rango: 3 meses-8.5 años). El desarrollo de enfermedad relevante fue significativamente mayor en el grupo de donantes ≥ 50 años (64 vs. 20.5%, p < 0.0001). En este grupo, la inmunosupresión potente –triple y cuádruple terapia– (p = 0.04) y el desarrollo de hepatitis aguda post-TH (p = 0.03) fueron las únicas complicaciones relevantes..cfg

ABSTRACT

Background: the natural history of recurrent hepatitis C after liver transplantation (LT) is extremely variable, with progression to allograft failure in a substantial proportion of patients. The identification of factors associated with this poorer outcome may improve results. While donor age has been identified as one of the most important factors, the actual options to modify this variable are limited.

Objectives: a) to describe the natural history of HCV (+) liver transplant recipients depending on donor age (< or ≥ 50 years), including clinical, biochemical, and histological outcomes; and b) to identify in the subgroup of organ recipients from aged donors (≥ 50 years) factors associated with an aggressive course.

Methods: a retrospective study of clinical and histological data including protocol liver biopsies for 162 HCV (+) liver transplant patients between 1997 and 2001 with at least one year of follow-up. Aggressive recurrent hepatitis C was defined as a progression to fibrosis > 1 during the first year post-LT, development of a cholestatic form of recurrent hepatitis C, and/or graft failure due to HCV during the first five years post-LT. Factors analyzed as potentially associated with recurrent hepatitis C included: a) recipient-related: demographics (age, sex), pre-transplantation (hepatocellular carcinoma, Child-Pugh classification, history of alcohol, HBV serological markers, antiviral treatment, nutritional status, biochemical variables); b) donor-related: demographics (age, sex), cause of death, grade of steatosis defined as minimal vs. moderate-severe > 10%; c) surgery-related: cold preservation and re-warming time, duration of procedure, blood transfusion; and d) post-LT management-related: immunosuppression, liver enzymes in the first 14 days post-LT, acute hepatitis post-LT, surgical complications (vascular and/or biliary).

Results: patients were divided into two groups according to donor age group 1 (< 50 years), n = 83, 51%, and group 2 (≥ 50 years), n = 79, 49%). Median follow-up was 5 years (range: 3 months-8.5 years). Aggressive recurrent hepatitis C occurred significantly more frequently in the older donor group (64 vs. 20.5%, p < 0.0001). In this group, potent immunosuppression –triple and quadruple regimens– (p = 0.04) and acute hepatitis post-LT (p = 0.03) were the only variables associated with aggressive recurrence. Degree of donor steatosis was not associated with the prognosis of recurrent hepatitis C.
variables asociadas con el desarrollo de hepatitis relevante. El grado de estatización del donante no se asoció con el pronóstico de la hepatitis C recurrente.

Conclusión: la utilización de donantes a años es, en parte, responsable de la progresión acelerada de la hepatitis tras el trasplante hepático. En caso de donantes a años, debe evitarse la sobre-immunosupresión y valorar la posibilidad de administrar tratamiento antiviral en los pacientes con hepatitis aguda recurrente.

Palabras clave: Trasplante hepático. Virus hepatitis C. Donantes. Cirrosis hepática.


BACKGROUND

Hepatitis C virus (HCV)-cirrhosis is the most frequent indication of liver transplantation (LT) (1). In these patients HCV recurrence occurs universally (2), resulting in the development of histologic hepatitis in the vast majority of recipients (3,4). The natural history of this hepatitis is highly variable with progression to cirrhosis in less than one year in some patients, while others have normal histology ten years after LT. Overall, progression to cirrhosis occurs in 10 to 30% within 3-5 years following transplantation (3,4). Based on the fibrosis progression rate it has been estimated that the time to the development of graft cirrhosis is approximately 9-12 years on average, a duration that is significantly shorter than that described in the immunocompetent population infected with HCV (1,5). In the medium-long term, recurrent HCV disease has a negative impact on survival, with survival rates lower than those obtained in patients transplanted for other causes (5-year survival of 60-70% in HCV-patients vs. 80% in other groups) (1,3-8). Moreover, preliminary data suggest that the histologic progression of recurrent hepatitis occurs at a faster rate, and hence the proportion of patients developing graft cirrhosis in the first years post-transplantation is greater among those who have been transplanted in more recent years (7). Predictive factors potentially associated with this worse outcome are the use of older donors as well as the introduction of more potent immunosuppressive agents. Other factors that have been implicated include the severity and early appearance of recurrent disease, infection with cytomegalovirus, and HCV genotype (4,5).

Based on these findings several strategies have been proposed in order to improve the outcome of these patients. These include avoidance of excessive immunosuppression (such as the use of boluses of steroids and/or OKT3), avoidance of abrupt changes in immunosuppression (particularly rapid steroid tapering) (9), use of prophylaxis against cytomegalovirus infection in HCV-recipients, and finally preferential use of young donors in HCV-infected recipients. While the first two proposals are potentially applicable in real practice, the use of “better” donors for HCV-patients seems a difficult choice to make not only due to ethical reasons but also due to unrealistic applicability, given the scarcity of young donors. In fact, in our own series only 48% of donors were younger than 50 years in the last two years.

Based on these findings, we performed this study whose main goals were: a) to describe the natural history of recurrent HCV in patients receiving grafts from donors ≥ 50 years of age, and in those receiving them from donors < 50 years of age, including the clinical, biochemical and histological course; and b) to identify in the group of older donors (≥ 50 years) other factors associated with poor outcome.

PATIENTS AND METHODS

Patients

Between January 1997 and May 2001, 224 adult patients underwent primary LT for HCV-related cirrhosis without HBV co-infection at our institution.

Inclusion criteria for the study were: a follow-up of at least 12 months (except in those with graft loss due to HCV recurrence) and lack of concomitant pathologies (such as de novo HBV infection or post-LT biliary problems) that could render histologic evaluation difficult. The presence of hepatocellular carcinoma was not a cause of exclusion; the criteria for acceptance in these patients are those described previously (7). Of the 224 patients initially evaluated, 162 fulfilled the inclusion criteria. There were 62 patients excluded due to: de novo HBV (n = 2), biliary problems (n = 5), death during the first year post-LT non related to HCV (n = 43), and/or lack of protocol liver biopsies or difficulties in histological evaluation (n = 12). Our institution followed a strict policy so that yearly protocol liver biopsies were performed in HCV-recipients allowing for a detailed follow-
up not only from a clinical point of view but also from a histologic perspective. Follow-up was at least 12 months in all patients (except those with aggressive recurrent hepatitis C or cholestatic hepatitis, or those who died from HCV-related disease), with a median of 5 years (range 3 months-8.5 years) since transplantation. Follow-up ended at the time of patient death, re-transplantation, and/or end of follow-up for the present study (May 2005).

**Histologic evaluation**

Protocol liver biopsies were done at yearly intervals. Additional liver biopsies were performed when clinically indicated.

All biopsies were reviewed by a single pathologist (JMR) and stained routinely. Biopsies were classified as hepatitis by evaluating both the degree of necro-inflammatory activity and stage of fibrosis, as previously described (6,10). To summarize, necro-inflammatory was calculated by combining the scores obtained for perportal necrosis (0 to 6), lobular degeneration and necrosis (0 to 4), and portal inflammation (0 to 4); total grading was as follows: 1 to 2, minimal; 3 to 6, mild; 7 to 10, moderate; 11 to 14, severe. The fibrosis score was: 0, none; 1, fibrous portal expansion; 3, bridging fibrosis, and 4, cirrhosis.

Aggressive recurrent hepatitis C was defined as progression to fibrosis > 1 during the first year, cholestatic fibrosing hepatitis, and/or graft failure during the first 5 years post-LT due to recurrent hepatitis C. We chose the cut-off of fibrosis ≥ 1 in the first year liver biopsy since it has been associated with progressive recurrent HCV disease in prior studies.

Pre and post-rewarming liver biopsies were done routinely in order to evaluate the quality of the liver and the degree of steatosis with the following score: 1 or minimum: < 10% of the hepatocytes with steatosis, 2 or mild: 11 to 20%, 3 or moderate: > 21-30%, 4 or severe: steatosis > 30%.

**Immunosuppression**

Induction immunosuppression was variable depending on the trial in which the patient was included, and on renal and brain function both before and immediately after transplantation, and was as follows: a) tacrolimus + steroids (n = 75); b) cyclosporine + steroids (n = 19); c) tacrolimus + mycophenolate mofetil + daclizumab (n = 3); d) cyclosporine + steroids + basiliximab/daclizumab (n = 13); e) tacrolimus + daclizumab (n = 4); f) tacrolimus + sirolimus + steroids (n = 1); g) tacrolimus + steroids + basiliximab/daclizumab (n = 4); h) cyclosporine + azathioprine + steroids (n = 31); i) atgam + cyclosporine or tacrolimus + steroids (n = 3); j) tacrolimus + azathioprine + steroids (n = 7); and k) atgam + steroids + cyclosporine + azathioprine (n = 2). Initial doses were as follows: a) **steroids**: methyl-prednisone given intravenously with dose tapering from 200 mg the first day post-LT to 20 mg of prednisone orally at day 7. The way prednisone was further tapered has been variable, and while in some patients tapering was done at a slow rate until withdrawal after 12 months of follow-up, in others the drug was withdrawn within 6 months (n = 26); b) **cyclosporine**: trough levels of 250-350 ng/ml during the first month; 150-250 ng/ml the second and third months; 100-150 ng/ml until the end of first year and about 50-100 ng/ml thereafter; c) **tacrolimus**: trough levels of 5-15 ng/ml the first 3 months, 5-10 ng/ml thereafter; d) **mycophenolate mofetil**: administered at 1 g/12 h orally; e) **sirolimus**: administered to obtain blood trough levels between 4-11 ng/ml; f) **basiliximab**: 20 mg intravenously the day of transplantation and the 4th postoperative day; and g) **daclizumab**: 2 mg/kg after hepatectomy and 1 mg/kg between the 7th and 20th days.

Long-term immunosuppression has been modified over time. In fact, in more recent years second line immunosuppressive agents such as mofetil mycophenolate or steroids have been tapered and withdrawn at earlier time-points, generally during the first 6 months post-LT.

Decisions regarding changes in immunosuppression depending on graft function were not homogenous over the years, hence no detailed analysis was feasible. It must be stated, though, that there has been a trend to avoid excessive immunosuppression in patients developing recurrent hepatitis C with dose reductions in their immunosuppressants –cyclosporine, tacrolimus, and steroids.

Potent immunossuppression was defined as the use of triple and quadruple regimens at full doses.

**Cytomegalovirus prophylaxis**

Gancyclovir, administered either intravenously for 14-21 days or orally (1 mg/8 h) for 90 days, or valgancyclovir 900 mg/day orally for 90 days, was given under the following circumstances: a) positive donor and negative recipient; b) re-transplantation; c) use of monoclonal or polyclonal antibodies; and d) surgery complicated with high blood-product requirements.

**Factors predictive of aggressive recurrent HCV disease and/or patient/graft survival**

In order to evaluate whether donor age determines a different course, patients were divided into two groups: those who received grafts from donors younger than 50 years (group 1) and those who received grafts from donors older than or at 50 years (group 2). Variables analyzed potentially associated with aggressive recurrent hepatitis C were those related to recipient, donor, surgery, immunosuppression, and/or variables from early post-transplantation period (Table I).
In a sub-group of patients (those transplanted between 1999 and 2001) other variables were analyzed in order to determine the economical implications associated with the use of old donors. These included causes of death, rate of readmission, number of days in the Intensive Care Unit, and number of hospitalization days.

The HCV genotype was not considered a potential predictive factor because a vast majority of patients are genotype 1b at our institution. Viral load was not evaluated because of missing data and changes in virological tests over time.

Retransplantation

Retransplantation was not considered in patients who developed graft cirrhosis during the first year post-LT, or in those over 65 years.

Statistical analyses

Patients were divided into two groups according to donor age (<50, ≥50 years). Categorical data were compared using a χ² test or Fisher’s exact test when indicated. When categorical variables were ordered, comparisons were done using a χ² for trend. Continuous variables were expressed as medians ± ED, and comparisons were done using Student’s t test. Variables that were not adjusted to normality were expressed as median and range, and compared by using the Mann-Whitney test. A p value ≤ 0.05 was considered statistically significant. All statistical analyses were performed with the SPSS 9.0 (SPSS Inc., Chicago, IL).

RESULTS

Baseline features in both groups of patients according to donor age

Fifty percent of patients (83/162, 51%) received a graft from a donor of less than 50 years (group 1). The remaining patients (n = 79, 49%) received a graft from an older donor (≥ 50 years) (group 2). Baseline features are summarized on table II. As expected, both groups were similar except for donor body mass index and presence of steatosis (11).

Natural history of recurrent hepatitis C.

Development of aggressive recurrent hepatitis C

The development of aggressive recurrent HCV disease occurred significantly more frequently in the group of aged donors than in the group of younger donors. Sixty-four percent of patients in the former group developed an aggressive recurrence as opposed to only 20.5% in the latter group. Other complications such as surgical problems and primary graft non-function were also more frequent in the group of aged donors when compared to group 1, but without reaching statistical significance (Table III).

Factors implicated in the histological course of recurrent HCV

Factors statistically significant (p < 0.05) or near statistical significance (p < 0.1) associated with aggressive recurrent hepatitis C in both groups and separately (group 1 and 2) are shown in table IV.
Economic implications

Both the rate of readmissions to hospital and the number of days in the Intensive Care Unit were significantly greater in the group of aged donors than in group 1 (Table V). Immunosuppressive treatments and transfusion of blood products were similar in both groups (Table II).

DISCUSSION

Recurrent hepatitis C after LT is a major problem in liver transplant units due to its high morbidity and mortality. One of the variables associated with an accelerated progression is the use of marginal grafts such as those derived from old donors (1,3,12). In the present study we confirmed this finding, similar to that described in the immunocompetent patient, where age at infection is one of the strongest factors implicated in the progression of chronic hepatitis C (13).

Although the indication of LT for HCV-related cirrhosis remains well established, there is a need to find solutions to the high mortality seen in this group of patients. Clearly, the best strategy would be to transplant these patients after clearing the virus; unfortunately, and in contrast to what has been achieved for hepatitis B, there are no effective antivirals that can be used safely prior to transplantation in HCV-infected patients. Given the association between donor age and course of recurrent hepatitis C, one potential strategy would be to target young donors to recipients infected with HCV. However, this alternative is difficult to apply not only because of ethical issues but also because of its likely low applicability. Indeed, in the last few years there has been a dramatic change in the pool of donors, with a reduction of those due to accidents and an increase in those owing to cerebro-vascular complications (3,12). These changes have led to an increase in donor age of about 10 years in the...
last decade, a circumstance that is likely to continue in the following years (3,12).

There are different theories that try to explain the reasons of liver aging including the progressive alteration of genetic messages, mitochondrial oxidative stress, loss of telomeres, and/or changes in immune responses (14-16). Moreover, elderly livers have macroscopic changes such as a reduction in their size and a change of color (darker); in addition, microscopically they have more lipofuscin, more steatosis, iron overload, and also fibrosis and inflammation without any known cause (14-16). All these changes together with the recurrence of HCV probably lead to a more aggressive course of hepatitis C in liver transplant recipients (17,18).

Another strategy that can be proposed to improve the outcome of HCV-infected recipients is to determine factors predictive of outcome, particularly in recipients of grafts from older donors, mainly factors that are present before liver transplantation or that develop in the early post-transplant period. Based on these associations, one potential strategy would be to use antiviral therapy in those who fulfilled criteria of poor prognosis; this was the main goal of our study. The major findings can be summarized as follows: in the group of old donors, acute hepatitis in the first months post-transplantation, use of triple and quadruple regimens at full doses, and shorter duration of prednisone were associated with a worse histological course of recurrent HCV. These findings confirm the results of earlier studies, including those from our own group (9,19), suggesting that a more rational use of immunosuppression, avoiding excessive immunosuppression and abrupt changes in the doses or the type of immunosuppressive agents, might be the key to improve outcomes, as we have recently described in a prospective study (9).

Surprisingly, steatosis does not seem to play an important role in the course of recurrent hepatitis C, both in those receiving grafts from younger or older donors. This finding is relevant since a prevalence of steatosis > 30% is about 10% in the general population (11). While other studies have observed a trend to lower survivals together with a poorer initial function and a greater incidence of primary graft non-functioning when using moderate to severe steatotic donors (> 30%) (17,20,21), we defined “relevant steatosis” using a cutoff of “> 10%” because the number of liver biopsies with steatosis > 30% was practically null (n = 3). The mechanisms through which donor steatosis may result in post-transplantation problems is still unclear, but may be due to a greater susceptibility to ischemia-reperfusion. Indeed, the worse outcome observed when using steatotic donors occurs in all transplant indications and typically refers to short-term survival (17,20,21). In addition, it has been hypothesized that, like in the immunocompetent patient, donor steatosis might result in a faster progression of hepatitis C in the long term. In our study, though, focused on the subgroup of recipients who received grafts from older donors, steatosis did not have a major role in determining an aggressive course of recurrent HCV. It is likely that additional factors associated with aging have more relevance, independently of steatosis degree. In addition, in our series, the small number of patients with moderate (between 20 and 30%; n = 4) or severe (> 30%; n = 3) steatosis could be also responsible for the lack of association.

In conclusion, the use of older donors results in a worse histologic progression of recurrent hepatitis C post-LT. This worse outcome has economical implications related to a greater rate of hospital admissions and longer hospitalizations. If aged donors are used, and given the improved results obtained with antivirals in recent years (22), a potential strategy proposed for HCV-recipient, particularly for those with high transaminase levels who develop acute hepatitis in the first months post-LT, could be to treat these patients with antiviral agents. In addition, the use of potent immunosuppression with triple or quadruple regimens at full doses should be avoided.

**REFERENCES**


**Table V. Hospitalization depending on donor age**

<table>
<thead>
<tr>
<th>Group 1: Donors &lt; 50 years (n = 83)</th>
<th>Group 2: Donors ≥ 50 years (n = 79)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days in Intensive Care Unit</td>
<td>2 (1-31)</td>
<td>3 (1-15)</td>
</tr>
<tr>
<td>Initial hospitalization duration (in days)</td>
<td>17 (1-137)</td>
<td>17 (2-210)</td>
</tr>
<tr>
<td>Number of hospital re-admissions</td>
<td>1 (0-6)</td>
<td>1 (0-6)</td>
</tr>
</tbody>
</table>

*All liver and kidney variables analyzed (AST, ALT, GGT, FA, bilirubin, platelets, prothrombin time, tacrolimus and cyclosporine trough levels, cholesterol, urea, creatinine) did not reach statistical significance, except for a trend in higher levels of tacrolimus among old donors.*