Effect of Calcineurin Inhibitors in the Outcome of Liver Transplantation in Hepatitis C Virus-Positive Recipients

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Background. There is a paucity of good studies evaluating the impact of calcineurin inhibitors on posttransplantation outcome in hepatitis C virus (HCV)-infected liver transplant (LT) recipients.

Methods. We sought to determine whether there are differences on posttransplantation survival and histologic recurrence in HCV-LT recipients based on initial immunosuppression (IS) by conducting a prospective study comparing tacrolimus (Tac) versus cyclosporine-based IS in patients undergoing LT between 2001 and 2007. Protocol liver biopsies were performed.

Results. Baseline characteristics (demographics, liver function at LT, genotype distribution, donor, surgery, and IS except for the type of calcineurin inhibitor) did not differ between groups. Severe disease (defined as bridging fibrosis, cirrhosis, cholestatic hepatitis, or allograft loss or death because of recurrent disease in the first year) was present in 67 of 253 (26.5%) and was equally distributed in the CsA and Tac groups (27% vs. 26%; $P=0.68$). Two thirds of protocol biopsies performed at 1 year showed some fibrosis without differences between CsA and Tac groups (75% vs. 70%). Advanced fibrosis (bridging fibrosis and cirrhosis) was diagnosed in 30% CsA and 24.5% Tac patients (P=NS). No differences in survival at 1 and 7 years were observed (83% and 67% vs. 78% and 64%, respectively, $P=0.4$). In summary, in patients undergoing LT for HCV-related liver disease, posttransplantation outcome is not related to the calcineurin inhibitor used.

Keywords: Hepatitis C recurrence, Immunosuppressant therapy, Cyclosporine, Tacrolimus, Steroids, Liver transplantation.

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The course of recurrent hepatitis C virus (HCV) is aggressive in one third of liver transplant (LT) recipients. Together with donor age, immunosuppression (IS) is one of the determinant factors influencing the natural history of recurrent HCV disease (*1–4*). In recent years, new immunosuppressive drugs with greater potency, designed to obtain a higher rate of graft tolerability with fewer side effects, have been implicated in the worsening in the severity of recurrent HCV observed in several centers (*2–4*). Although existing data demonstrate an association between the "state of IS" and posttransplantation HCV disease progression, the exact effect of the different agents is still unclear (*2–4*). An understanding of these potential associations would result in the proposal of strategies to improve outcome based on modifications of IS (*5*).

Using the HCV subgenomic replicon system, an inhibitory effect of cyclosporine on HCV protein expression and RNA levels was described recently (*6*). This effect was not detected with tacrolimus (Tac). Whether this in vitro data translate into differences in the clinical arena is still a matter of controversy. In most retrospective studies, no differences between CsA and Tac regimen have been reported (*1–4*). Furthermore, no differences in patient or graft outcome were reported in a recent meta-analysis (*3*). Finally, in our own initial experience based on a prospective study comparing

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CsA with Tac-based IS, the posttransplantation histologic disease severity was similar regardless of the calcineurin inhibitor (*7*). However, these preliminary results were based on a small sample size and short follow-up. We describe here both the clinical and histologic outcome of a large sample of patients who have been immunosuppressed with CsA or Tac since 2001. Our initial hypothesis was that both patient or graft survival and severity of recurrent HCV would not depend on the type of immunosuppressive drug used during the first year posttransplantation. Therefore, the aim of this study was to determine whether there are differences in histologic and clinical outcome with regard to the calcineurin inhibitor used. To test this, we compared the rate of HCV severe disease and graft or patient survival between two groups of patients who have been prospectively treated in a pseudorandomized study with CsA or Tac in combination with prednisone or mycophenolate mofetil (MMF).

RESULTS

Patient Demographics

Two hundred fifty-three of 310 patients transplanted between 2001 and 2007 fulfilled the inclusion criteria (Table 1). The remaining 57 LT recipients were excluded because of negativity of HCV RNA after transplantation (n=1), associated medical conditions (Budd-Chiari, n=2), retransplantation (n=22), combined kidney transplantation (n=4), HIV infection (n=8), hepatitis B virus infection (n=8), violation of protocol (n=11), and lost to follow-up (n=1).

Comparison Between the "Cyclosporine" and "Tacrolimus" Groups

The treatment of the 253 patients was CsA based in 136 patients and Tac based in 117 patients. There were no differences in baseline characteristics, including patient and donor demographics, pretransplantation features, genotype distribution, surgical-related factors, and IS in addition to the calcineurin inhibitor used (Table 1). In summary, this was a population of mostly male LT recipients with a median age of 56.5 years, a coexistent hepatocellular carcinoma (HCC) at transplantation in a significant proportion of patients (43%), with few having received antiviral therapy in the past (15.5%), and mainly infected with HCV genotype 1 (83%). The donor age was 53 years. Concomitant IS with MMF or monoclonal antibodies was used in a small proportion of patients (15% MMF, 7.5% interleukin-2 receptor antibodies). In addition, only 8% of patients received a steroid-free regimen. Finally, few patients required switching the primary calcineurin inhibitor during follow-up (~4%).

TABLE 1. Baseline characteristics of the study population

	CsA-based IS (n=136)	Tac-based IS (n=117)	*P*
Gender (% male)	103 (76)	81 (69)	0.24
Age (yr), median (range)	56 (26–67)	57 (33–67)	0.38
HCC (%)	63 (46)	48 (41)	0.39
Alcohol (%)	38 (28)	32 (27)	0.9
Child C classification (%)	57 (42)	56 (48)	0.2
MELD UNOS (n=57)	14 (7–29)	15 (7–29)	0.7
Pre-LT diabetes (%)	29 (21)	17 (14.5)	0.16
Pre-LT arterial hypertension (%)	16 (12)	15 (13)	0.8
Pre-LT acute renal failure (%)	11 (8)	10 (8.5)	0.9
Pre-LT antiviral therapy (%)	20 (15)	19 (16)	0.7
Genotype (% 1a/1b)	77 (83)	67 (83)	0.98
Time in waiting list (d)	84 (0–550)	110 (1–437)	0.09
Donor gender (% male)	81 (60)	65 (56)	0.5
Donor age (yr), median (range)	51 (12–83)	56 (15–81)	0.15
Donor hypotension (%)	50 (36.5)	45 (38)	0.2
Days in intensive care unit	3 (1–82)	4 (1–80)	0.8
Warm ischemia time (min)	40 (15–340)	40 (15–225)	0.8
Cold ischemia time (min)	302 (22–720)	325 (30–850)	0.7
Initial severe graft dysfunction (%)	8 (6)	10 (8.5)	0.1
Biliary complication	27 (20)	18 (15.5)	0.4
Use of prostaglandins (%)	27 (20)	26 (22)	0.6
Steroid-free IS (%)	10 (7.5)	9 (8)	0.9
Initial treatment with MMF (%)	25 (18)	15 (13)	0.2
Induction with monoclonal antibodies (%)	12 (9)	7 (6)	0.3
Acute rejection (%)	10 (7.5)	7 (6)	0.8
Switched to Tac/Cyclosporine (%)	7 (5)	4 (3.5)	0.7

Tac, tacrolimus; HCC, hepatocellular carcinoma; ICU, intensive care unit; LT, liver transplantation; IS, immunosuppression; MMF, mycophenolate mofetil; MELD, model for end-stage liver disease; UNOS, United Network for Organ Sharing.

Clinical Outcome in Those Under Tacrolimus- Versus Cyclosporine-Based Immunosuppression

Histologic Recurrent Disease

The percentage of patients with the first-year liver biopsy available was not statistically different between groups (85/136 vs. 79/117, $P=0.4$). There were no differences in the reasons for lacking a first-year biopsy. The most common reason for the lack of the protocol liver biopsy was early death unrelated to HCV before reaching 1-year follow up (n=35, CsA 17, Tac 18). Lack of patient consent or administrative problems were the second cause in 31 patients. Of these, 15 patients underwent subsequently protocol liver biopsies at 2 or 3 years posttransplantation. A previous diagnosis of aggressive HCV had been made in 17 patients without further histologic follow-up before starting antiviral therapy. These biopsies were used for the analysis of histologic outcome. Finally, a non-HCV–related diagnosis was made in seven cases (lymphoproliferative disorder, n=1; acute cellular rejection, n=2; chronic rejection, n=1; and biliary problem, n=3), and these biopsies were not used for the analysis. A flow chart of the study population is shown in Figure 1. Overall, 181 liver biopsies performed within the first year (90 CsA and 91 Tac) were used in the analysis of histologic outcome at 1 year. None of these biopsies were performed after starting antiviral therapy. The rate of severe hepatitis with bridging fibrosis or cirrhosis in the first-year liver biopsy was similar in the CsA versus Tac group (30% vs. 24%). In addition, the percentage of patients developing cholestasis-like hepatitis was also sim-

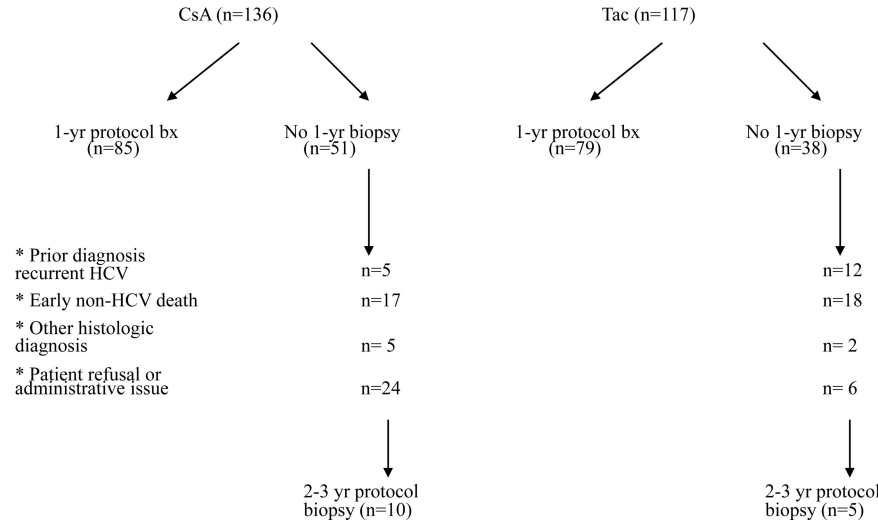

FIGURE 1. Flow-chart of study population. Note: the analysis of 1-year histologic outcome was based on 90 and 91 biopsies performed within the first year in the CsA and Tac group, respectively (eighty-five 1-year protocol liver biopsies+5 with prior diagnosis of recurrent HCV in clinically indicated liver biopsies in the CsA group and seventy-nine 1-year protocol liver biopsies+12 with prior diagnosis of recurrent HCV in clinically indicated liver biopsies in the Tac group). CsA, cyclosporine A; HCV, hepatitis C virus; Tac, tacrolimus; bx, biopsy.

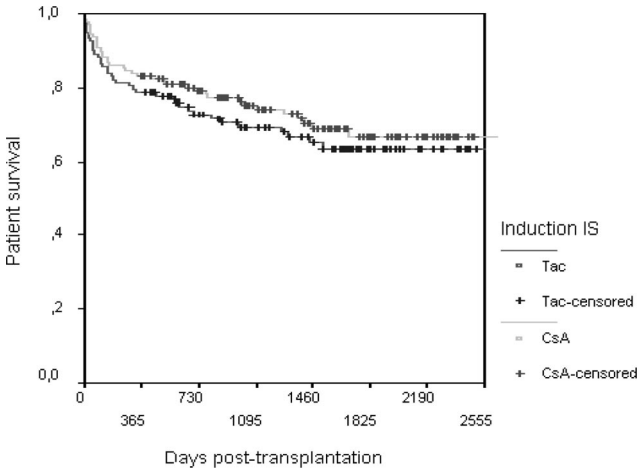

FIGURE 2. Patient survival in cyclosporine-versus tacrolimus (Tac)-based immunosuppression (IS). Patient survival at 1, 3, 5, and 7 years was similar in both groups; CsA: 83%, 75%, 67%, and 67% versus Tac: 78%, 70%, 64%, and 64% (log-rank comparison of treatment groups, $P=0.4$).

TABLE 2. Outcome of Neoral Cyclosporine vs Tac-based immunosuppression patients

	CsA-based IS (n=136)[a]	Tac-based IS (n=117)[a]	*P*
Primary endpoint (CH±F=3–4±graft loss or patient death because of HCV within 1 yr from LT)	37 (27%)	31 (26%)	0.68
F>0 in the first year biopsy	68/90 (75%)	63/91 (69%)	0.8
F=3–4 in the first year biopsy	27/90 (30%)	22/91 (24%)	0.37
Cholestatic hepatitis	9/90 (10%)	10/91 (11%)	0.8
F=0 in the first year biopsy	22/90 (25%)	27/91 (30%)	0.8
F=3–4 in the last available biopsy	42/110 (38%)	36/97 (37%)	0.8
Time to last biopsy (d)	775	712	0.3
Antiviral therapy	40 (29%)	29 (25%)	0.4
SVR to antiviral therapy	14/37 (38%)	11/28 (39%)	0.9
Allograft loss	43 (32%)	40 (34%)	0.6
Death	37 (27%)	37 (31%)	0.4
Recurrent HCV disease	12	14	
Related with IS	13	12	
Sepsis	12	10	
De novo tumour	0	2	
Chronic rejection	1	0	
Recurrent HCC	4	3	
Others	6	6	
Technical complication	2	2	

[a] Ninety and 91 liver biopsies within 1 year were available for the analysis of histologic outcome in the CsA and Tac group, respectively.

"Others": cardiovascular deaths (n=5), severe pancreatitis (n=1), traumatism (n=1), renal insufficiency (n=1), neurological complications (n=3), and gastrointestinal bleeding (n=1).

CH, cholestatic hepatitis; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; IS, immunosuppression; F, fibrosis stage; LT, liver transplantation; SVR, sustained virologic response; Tac, tacrolimus.

ilar in both groups (10% vs. 11%). No differences were observed in the percentage of biopsies showing "fibrosis zero" at 1 year posttransplantation (24% vs. 30%; $P=0.4$). Overall, two thirds of patients (75% in the CsA group and 70% in the Tac group) had some fibrosis at 1 year posttransplantation.

Patient and Graft Survival

Graft and patient survival did not differ between the two groups (Fig. 2 and Table 2). Patient survival at 1, 3, 5, and 7 years was 83%, 75%, 67%, and 67% in the CsA group and 78%, 70%, 64%, and 64% in the Tac group, respectively (log-rank comparison of treatment groups, $P=0.4$). The causes of death were also similar with recurrent disease and sepsis accounting for the majority of deaths in these patients (32% and 32% in the CsA group vs. 38% and 27% in the Tac arm). Only seven patients were retransplanted because of recurrence of HCV disease, four in the CsA group (3%) and three in the Tac group (2.5%). Approximately 25% of patients in each group received antiviral therapy during the follow-up with a sustained viral response of 38% to 39% that did not differ between groups (Table 2).

Primary Endpoint

The percentage of patients reaching the primary endpoint (fibrosing cholestatic hepatitis±bridging fibrosis or cirrhosis±graft loss or patient death because of HCV within 1 year from LT) was not statistically different between groups (27% for CsA vs. 26% for Tac; $P=0.68$; Table 2).

DISCUSSION

Recurrent HCV is a major problem in LT units because of both its high frequency and potential aggressive nature. IS is one of the key factors determining patient and allograft outcome (*1–4, 23, 24*). Indirect findings that support the importance of IS in the natural history of HCV include the greater rate of fibrosis progression in immunosuppressed populations, such as LT recipients or HIV coinfected patients compared with immune-competent individuals, and the known detrimental effect of high methylprednisolone boluses on the severity of recurrent HCV. In addition, some centers have reported worse outcomes in HCV-LT recipients in recent years paralleling the introduction of more potent immunosuppressive agents (*8*), thus raising the question to whether these new agents might be implicated in this aggressive pattern. Unfortunately, there is a lack of studies assessing the specific effect of each immunosuppressive agent on both viral replication and disease progression (*7*, *9–11*). In fact, most of the information comes from retrospective studies where protocol liver biopsies are generally missing (*1–4*). A potential antiviral effect of CsA was recently suggested based on in vitro studies (*6*). Since 2001, our center policy has been to prospectively treat the transplant patients with CsA or Tac in combination with steroids or MMF. Preliminary results using only HCV-positive patients were published in 2006 and showed no major differences in outcome between both groups (*7*). We present here the final results of this prospective study with a significantly larger sample size. The major conclusions may be summarized as follows: (1) CsA-based IS is similar to Tac with regard to the severity of recurrent standard chronic HCV; (2) severe hepatitis with a cholestatic pattern occurs at the same rate, regardless of the calcineurin inhibitor used; and (3) there are no differences in patient or graft survival that depend on the calcineurin inhibitor used.

We chose to evaluate the histologic outcome at 1 year because it has become increasingly common to start antiviral therapy based on the results of the first-year protocol liver biopsy (*2*). Several studies have indeed reported that the first-year histologic findings, in particular the stage of fibrosis and the degree of necroinflammation, can be helpful in predicting a progressive HCV disease (*12–18*). In fact, the studies have shown that progression of disease is unlikely to occur in patients without evidence of fibrosis in their first-year liver biopsy, and on the contrary, the presence of liver fibrosis expanding beyond the portal tract 1 year after transplantation identifies patients at high risk of progressive disease and graft loss. In a prior study, cirrhosis developed in 3 of 35 (8.6%) patients with no fibrosis in the first-year liver biopsy when compared with 6 of 22 (27%) patients with a fibrosis score of 1 or more (*13*). In another study, the cumulative rate of remaining free of allograft decompensation was greater than 80% in those with mild fibrosis in a baseline liver biopsy performed at 3 or 12 months, whereas it dropped to less than 50% in those with fibrosis more than or equal to 2 (*15*). In this study, approximately two thirds of patients, with no differences between CsA and Tac, had some fibrosis in their first-year liver biopsy, suggesting that these should be the targets of antiviral therapy given their higher risk of progressive HCV disease.

In addition, we also assessed other medium and long-term endpoints, such as "use of antiviral therapy," patient and graft survival, or progression to bridging fibrosis and cirrhosis within 5 years from transplantation, without finding significant differences between the two groups either. Mortality in this cohort was significant, approximately 30%, but without significant differences between groups. Most of the deaths were because of infectious complications and recurrent disease, as pointed out by other authors (*19*, *20*).

Our results are in accordance to many studies which, although not specifically aimed at evaluating the effect of these compounds on histologically assessed HCV-related disease progression, found no differences in the overall outcome (*1–4*, *7*, *9–11*). Our findings also support the results from a recent meta-analysis that evaluated the impact of calcineurin inhibitors on patient and graft survival (*3*).

We did not find differences in the rate of sustained viral response based on the calcineurin inhibitor used. We cannot assure although these were the immunosuppressive agents that patients were taking at the time of antiviral therapy because this study was not aimed at evaluating the impact of calcineurin inhibitors on antiviral response. However, only a few patients in each group had their primary immunosuppressive agent switched during follow-up.

The major limitation of this study is its relatively small sample size. Although it is the largest prospective study to assess the recurrence of HCV based on initial calcineurin inhibitor, it is still inadequate from a statistical point of view. Indeed, the statistical power of this study was approximately 6% with an absolute risk difference between CsA and Tac of −3.3% with a 95% confidence interval ranging from −14% to 7.5%. Although this wide confidence interval may be due to real lack of differences between groups, it is more likely that it is related to the "statistically inadequate sample size." Indeed, to test our hypothesis, the perfect study would need to include 1192 patients per group to detect a difference of 5% in the primary endpoint with a statistical power of 0.80. Larger and probably multicenter studies would need to be performed to confirm our data.

In conclusion, the results from this large prospective study confirm previous assumptions that the choice of calcineurin inhibitors does not impact the histologic severity of recurrent HCV, at least in the short-term, or long-term patient and graft survival. It is likely that progression of HCV disease is more a reflection of overall excess IS or the way we modify the different immunosuppressive drugs during the first months posttransplantation than the direct effect of a specific immunosuppressive agent.

MATERIALS AND METHODS

Patients

Between October 2001 and December 2007, 310 HCV-infected adult patients underwent liver transplantation at our institution. The study was an open-label study, where all transplant patients were immunosuppressed with

CsA or Tac following the center policy. All patients gave appropriate informed consent to follow this policy. The center transplant committee, supervised by the ethics committee, reviewed and approved this clinical study. In essence, patients whose chart number was an odd number were immunosuppressed with Tac, whereas CsA was used in the case of charts with even numbers. Inclusion criteria were transplantation for HCV-related end-stage liver disease with positive HCV viremia posttransplantation receiving initial IS with CsA or Tac. Patients with the following exclusion criteria were not used for this analysis: violation of protocol, hepatitis B virus coinfection, HIV infection, negativity of HCV-RNA posttransplantation, combined kidney or lung transplantation, or undergoing liver retransplantation for reasons other than primary allograft dysfunction. Standard or pegylated interferon in combination with ribavirin was used for the treatment of histologically proven recurrent HCV, generally evaluated in the first-year liver biopsy. Antiviral therapy was initiated at earlier time points in cases of aggressive recurrent disease, defined as cholestatic hepatitis or progressive fibrosis detected in sequential liver biopsies (*21*). The data regarding the efficacy and tolerability of antiviral therapy in our setting have already been published in prior studies (*21*). The criteria used for selecting patients with cirrhosis and a localized HCC are those proposed previously (*22*). The follow-up of this analysis was terminated at the time of the patient's death, retransplantation, or at the end of the observation period (January 2009).

Histologic Assessment

Protocol liver biopsies were performed 1 year (±4 months) posttransplantation. The following protocol biopsies were performed at 1 or 2 years interval depending on initial histologic findings and the use and results of antiviral therapy. Additional biopsies were performed when clinically indicated. All biopsy specimens were reviewed by a single pathologist (J.M.R.) in a blinded fashion, and for the histologic part of the study, only those obtained before any antiviral therapy was instituted were evaluated. Sections were stained routinely with hematoxylin-eosin, reticulin, Perls' and Orcein stains.

Liver biopsies classified as "hepatitis" were scored evaluating both the stage of fibrosis and the degree of necroinflammatory activity. The grade was determined by combining the histologic activity index scores for periportal necrosis, lobular degeneration, and necrosis and portal inflammation and was defined as follows: 1 to 2, minimal; 3 to 6, mild; 7 to 10, moderate; and 11 to 14, severe. The stage corresponded to the original histologic activity index fibrosis score: 0, none; 1, fibrous portal expansion; 3, bridging fibrosis; and 4, cirrhosis. Cholestatic hepatitis was defined following recent recommendations (*2*).

Immunosuppression

During the study period, all patients undergoing LT at our institution were prospectively treated with "CsA neoral+steroids" or "Tac+steroids." This pseudorandomized trial used the chart number for treatment allocation. Additional therapies were used in cases of early posttransplantation complications (renal or neurological impairment) that required a substantial reduction in calcineurin inhibitor doses. In these cases, interleukin-2 receptor antibodies or, more frequently, MMF were used and calcineurin inhibitors were started several days after transplantation at reduced doses. Steroids were avoided and substituted by MMF in patients with pretransplantation diabetes. Initial doses were as follows: methylprednisolone given intravenously with tapering of the dose from 200 to 20 mg at day 6, at which time 20 mg/day of prednisone was administered orally; CsA (trough levels of 250–350 ng/mL the first month, 150–250 ng/mL the second and third months, 100–150 ng/mL until the end of the first year, and approximately 100 ng/mL thereafter); Tac (trough levels of 10–15 ng/mL the first month, 5–10 ng/mL the second and third months, and 3–10 ng/mL thereafter). MMF was dosed at 1 g/12 hr and adjusted based on side effects. Prednisone dose was started at 20 mg 1 week posttransplantation and tapered down at a slow rate with final withdrawal after 9 to 12 months. Only in cholestatic hepatitis or in patients with severe side effects related to corticosteroids, prednisone was tapered down more rapidly.

Cytomegalovirus Prophylaxis

Ganciclovir or valganciclovir were administered under the following circumstances: (1) positive donor and negative recipient; (2) retransplantation; (3) use of monoclonal or polyclonal antibodies; and (4) surgery complicated with high blood product requirements.

Outcome Variables

Progression to severe disease within the first year (bridging fibrosis, cirrhosis, cholestatic hepatitis, and death due to recurrent hepatitis) was used as the primary endpoint. Secondary endpoints included: (1) bridging fibrosis or cirrhosis diagnosed in the first-year liver biopsy, (2) cholestatic hepatitis, (3) no fibrosis in the first-year liver biopsy, and (4) graft/patient survival.

Factors Analyzed and Compared Between the Two Groups

These included (1) demographics: age, gender distribution and body mass index; (2) pretransplantation variables: presence of HCC, Child-Pugh classification, history of significant alcohol consumption, diabetes, arterial hypertension or acute renal failure, and history of antiviral therapy; (3) donor-related variables: age, gender, diabetes, and arterial hypotension; (4) surgical-related variables: duration of cold preservation and rewarming time, duration of intervention, initial graft function, and time in the intensive care unit; and (6) IS-related variables: histologically diagnosed rejection episodes requiring methylprednisolone boluses and use of additional immunosuppressive drugs.

Statistical Analyses

Categorical data were compared using a chi-square test or Fisher's exact test when indicated. When categorical variables were ordered, comparisons were done using a chi-square test for trend. Continuous variables were expressed as median and range and compared by the Mann-Whitney *U* test. HCV-related severe disease was defined by the presence of bridging fibrosis, cirrhosis, fibrosing cholestatic hepatitis, and death because of HCV occurring within the first-year posttransplantation. The log-rank test was used to compare patient and graft survival between the two groups. All statistical analyses were performed with SPSS version 9.0 (SPSS Inc., Chicago, IL).

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