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ORIGINAL ARTICLE

Predictors of Sustained Virological Response After Antiviral Treatment for Hepatitis C Recurrence Following Liver Transplantation

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Factors associated with sustained virological response (SVR) in patients treated for hepatitis C virus (HCV) recurrence after liver transplantation (LT) are unclear. Ninety-nine HCV-positive/hepatitis B surface antigen–negative patients received antiviral treatment (AVT) with interferon/peginterferon plus ribavirin for HCV recurrence after LT. Cyclosporine (CyA) or tacrolimus (TAC) was used as the main immunosuppressor in 37 (37%) and 62 (63%) patients, respectively. Twenty-five patients (25%) achieved an SVR. Twenty-seven donor-related, recipient-related, HCV-related, and immunosuppression-related variables were investigated for their association with SVR. In logistic regression analysis, donor age $<$ 60 years (odds ratio = 4.45, 95% confidence interval = 1.39-14.19, $P = 0.01$), viral genotype other than 1 (odds ratio = 4.97, 95% confidence interval = 1.59-15.48, $P = 0.006$), and the use of CyA during treatment (odds ratio = 6.85, 95% confidence interval = 2.15-21.73, $P = 0.001$) were predictors of SVR. Patients treated with CyA (SVR rate: 43%) and those treated with TAC (SVR rate: 14%) were comparable for all variables, except for a shorter ischemia time and shorter timing of AVT initiation in the TAC group ($P = 0.02$ and $P = 0.005$, respectively) and a greater use of anti-CD25 antibodies, azathioprine, and mycophenolate mofetil in the CyA group ($P = 0.03$, $P < 0.001$, and $P = 0.001$, respectively). The rate of AVT discontinuation due to side effects was similar between groups (16% versus 8%, $P = 0.3$). In conclusion, the type of immunosuppression during AVT may predict SVR in patients treated for HCV recurrence after LT. ***Liver Transpl 15:782-789, 2009.*** © **2009 AASLD.**

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Hepatitis C virus (HCV)–related cirrhosis is the leading indication for liver transplantation (LT) in both the United States and Europe.[1,2] Hepatitis C recurrence is almost universal, leading to severe liver damage in 30% of patients within 5 years of transplant.[3,4]

In patients with posttransplant HCV recurrence, antiviral treatment (AVT) with interferon (IFN) and ribavirin is the only way to prevent severe reinfection of the graft, even though sustained virological response (SVR) is achieved in a minority of cases.[5]

Although predictors of severe HCV recurrence have been identified in recent years,[5-11] factors associated with the probability of SVR are much less clear. In fact, the effectiveness of AVT may be partly influenced by the same factors determining the natural course of HCV recurrence—donor age, quality of the graft, HCV-RNA level, viral genotype, type of immunosuppression, occurrence of rejection, and occurrence of cytomegalovirus (CMV) infection—and partly linked to other variables, such as the type of AVT, patient conditions, and grading and staging of reinfection at the time of initiation of antiviral drugs.[5,12]

Primary immunosuppression and particularly the use of cyclosporine (CyA) or tacrolimus (TAC) have been

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AVT, antiviral treatment; BMI, body mass index; CI, confidence interval; CMV, cytomegalovirus; CyA, cyclosporine; HCV, hepatitis C virus; ICU, intensive care unit; IFN, interferon; PEG-IFN, pegylated interferon; LT, liver transplantation; MMF, mycophenolate mofetil; RBC, red blood cell; SVR, sustained virological response; TAC, tacrolimus.

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extensively studied because of the hypothesis that this could determine the course of posttransplant HCV recurrence.[13] CyA (and not TAC) has been shown to inhibit HCV replication in vitro,[14] but no clear advantages from using CyA instead of TAC have been observed in clinical settings.[13]

When focusing on the attainment of a response to AVT, however, some studies have shown that the use of CyA may produce better results than TAC.[15-17]

In the present study, we explored factors associated with SVR in patients treated for HCV recurrence after LT.

PATIENTS AND METHODS

Patients

From November 1998 to July 2006, 251 consecutive HCV-positive/hepatitis B surface antigen–negative patients underwent primary, isolated, whole LT at the Liver and Multiorgan Transplant Unit of the Department of Surgery and Transplantation at the University of Bologna.

Among them, 30 (12%) patients lost their graft for reasons unrelated to HCV recurrence within 3 months of transplant without receiving AVT and were excluded from the study.

Of the remaining 221 patients, 112 (51%) received AVT for HCV recurrence; 6 (3%) were still on treatment at the end of the follow-up, and 106 (48%) completed or definitively stopped AVT. Among the treated patients, 7 were human immunodeficiency virus–positive and/or developed cholestatic hepatitis.[8] Because of the lower expected response rate of the human immunodeficiency virus–positive population and the peculiarity of cholestatic hepatitis versus recurrent HCV, these patients were excluded from the study. The remaining 99 patients formed the study population.

There were 72 (73%) males and 27 (27%) females, with a median age of 56 (34-67) years. Forty (40%) patients were hepatitis B anti-core antibody–positive.

At LT, the median Model for End-Stage Liver Disease score,[18] without any additional score (ie, due to the presence of hepatocellular carcinoma), was 16 (range: 7-45).

HCV Detection and Genotyping

Quantitative HCV-RNA was routinely determined in all patients with a branched DNA assay (Quantiplex HCV 2.0, Chiron Corp). The lower limit of detection of the quantitative assay was 0.615 IU/mL. At the initiation of AVT, the median level of HCV-RNA was 2.40 (0.08-7.88) $\times$ 10^6 IU/mL.

The viral genotype was determined by nested reverse transcription polymerase chain reaction of the core region with type-specific primers (Inno-LiPA HCV, Innogenetics, Ghent, Belgium) and classified according to the criteria of Simmonds and colleagues.[19] Most patients (n = 65, 66%) had viral genotype 1.

Diagnosis of Hepatitis C Recurrence

In all 99 patients considered in this study, 3 criteria had to be fulfilled before initiation of AVT: (1) alteration of liver function tests in the absence of vascular, biliary, drug, or infectious causes; (2) liver biopsy confirming HCV recurrence; and (3) detectable quantitative HCV-RNA. In all patients, liver histology showing HCV recurrence was available within 2 months before AVT was started. Hepatitis recurrence was defined by a histology activity index $\geq$ 3.[20]

At the initiation of AVT, the fibrosis stage was 0 to 2 in 84 (85%) patients and 3 to 4 in 15 (15%) patients,[21] whereas the median level of aspartate aminotransferases was 119 (36-876) IU/L, and that of alanine aminotransferases was 163 (23-1010) IU/L.

All patients who died or lost their graft because of posttransplant hepatitis C had histological confirmation of recurrent disease.

Donor Characteristics and Operative Parameters

There were 65 (66%) male donors and 34 (34%) female donors; the median donor age was 60 (15-86) years. Four (4%) donors were HCV-positive. Cardiac arrest occurred before or during organ recovery in 8 (8%) cases, whereas noradrenaline was used to support hemodynamics in 39 (39%) cases. Marked alteration of donor liver function test (ie, aspartate and/or alanine aminotransferases $>$ 500 IU/L or bilirubin $>$ 2 mg/dL)[22] was observed in 7 (7%) cases. Graft macrovesicular steatosis $>$ 30% was present in 2 (2%) cases.

The median donor intensive care unit stay was 2.5 (0-14) days. The median ischemia time was 423 (175-812) minutes. The median intraoperative red blood cell transfusion requirement during LT was 2400 (300-25,910) mL.

Immunosuppression and Acute Cellular Rejection

CyA and TAC were the main immunosuppressive drugs used in this study population. The assignment to CyA or TAC was not dictated by a specific choice but simply reflected the increasing use of TAC as the primary immunosuppressive agent by most programs during the study period. In particular, 37 (37%) patients were administered CyA and 62 (63%) received TAC at the initiation of and during AVT. Serum levels of CyA were maintained between 90 and 150 ng/mL, and those of TAC were maintained between 4 and 10 ng/mL during AVT.

Corticosteroid tapering was completed within 6 months after transplant in 30 (30%) patients, whereas 69 (70%) patients were administered steroids for more than 6 months or never stopped them.

Anti-CD25 antibodies (basiliximab or daclizumab) or anti-CD52 antibodies (alemtuzumab) were used as induction in 10 (10%) and 3 (3%) patients, respectively,

whereas 25 (25%) patients received azathioprine for the first 6 months post-LT.

Mycophenolate mofetil (MMF) and rapamycin were used in 10 (10%) and 6 (6%) patients, respectively.

Steroid pulse doses to treat acute rejection (500-1000 mg of methylprednisolone) were administered at least once to 35 (35%) patients before or during AVT. Two (2%) patients required only monoclonal CD3-antibodies (OKT3) to treat steroid-resistant rejection.

CMV Immune Status and Infection

Immunity against CMV was determined in all recipients and donors before LT by the detection of immunoglobulin against CMV (Vidas-ELFA, Biomerieux, Italy). All patients were followed with repeated quantitative determination of pp65 antigenemia, and gancyclovir treatment was started if an increase in antigenemia was observed between 2 subsequent observations or in the presence of CMV disease.[23] CMV infection was defined by the need for gancyclovir treatment and occurred in 19 patients (19%).

Treatment of Hepatitis C Recurrence

AVT was potentially offered to all patients with clinical and histological evidence of HCV recurrence. No patients received preemptive AVT. The minimum duration of AVT was 24 weeks, regardless of the achievement of a complete virological and biochemical response during this period and unless adverse events contraindicating AVT occurred. After 2002, an attempt to treat patients with genotypes 1 and 4 for 48 weeks was routinely made. AVT was avoided or discontinued only in the event of uncontrollable side effects or clinical contraindications. SVR was defined as undetectable HCV-RNA for at least 24 weeks after cessation of AVT.

AVT was started with 1.5 MU of IFN α-2b 3 times weekly plus 400 to 600 mg of ribavirin daily for 1 to 2 weeks, and if it was tolerated, doses were increased to 3 MU of IFN α-2b 3 times weekly plus up to 1200 mg of ribavirin daily. In 2002, this regimen was replaced by 135 to 180 μg of pegylated interferon (PEG-IFN) α-2a or α-2b weekly plus weight-adjusted daily ribavirin. The choice between PEG-IFN α-2a and PEG-IFN α-2b mainly depended on the timing after LT of the initiation of AVT and on the general conditions of the patients. Some patients who could not tolerate PEG-IFN were switched to IFN α-2b, whereas others who initially did not respond to IFN α-2b were subsequently switched to PEG-IFN. Accordingly, 61 (62%) patients received IFN α-2b only, 32 (32%) received PEG-IFN only, and 6 (6%) were sequentially administered either IFN α-2b or PEG-IFN.

IFN doses were reduced when the neutrophil count was lower than 800 cells/μL and/or the platelet count was lower than 50,000/μL. IFN was stopped when the neutrophil count was lower than 500 cells/μL and/or the platelet count was lower than 50,000/μL. Granulocyte colony stimulating factor was used when the neutrophil count was lower than 800 cells/μL.

Ribavirin dose reduction was considered when the hemoglobin level was lower than 10 g/dL, and it was stopped when the hemoglobin level was lower than 8 g/dL. Erythropoietin was administered when the hemoglobin level was lower than 9 g/dL.

The following variables were investigated for their hypothetical association with SVR: donor gender, age (≤60 versus >60 years), HCV status (positive versus negative), use of noradrenaline (yes versus no), transaminases > 500 U/L and/or bilirubin > 2 mg/mL (yes versus no), intensive care unit stay (≤7 versus >7 days), ischemia time (≤8 versus >8 hours), and red blood cell transfusions during LT (≤8 versus >8 L); recipient gender, age (≤55 versus >55 years), body mass index (≤25 versus >25), viral genotype (1 versus others), timing after LT of AVT (≤6 versus >6 months), HCV-RNA levels (≤2.4 versus $>2.4 \times 10^6$ IU/mL), transaminase levels (≤3 versus >3 × upper normal limit), and fibrosis stage (0-2 versus 3-4) at the initiation of AVT; main immunosuppressive drug at the initiation of AVT (CyA versus TAC), duration of steroid tapering (≤6 versus >6 months), use of monoclonal antibodies (yes versus no), MMF (yes versus no), rapamycin (yes versus no), and azathioprine (yes versus no); and occurrence of post-LT CMV infections (yes versus no), administration of steroid boluses (yes versus no), and type of IFN for AVT (IFN α-2b versus PEG-IFN versus both).

Graft macrovesicular steatosis > 30% and use of OKT3 were not included in the analysis because each of these factors was present in 2 cases only, as reported previously.

Statistical Analysis

Results were expressed as prevalence or as median and range of values. Differences between continuous and categorical variables were calculated with the Mann-Whitney U test and the χ^2 test or Fisher's exact test, respectively. Graft survival was calculated from the date of LT to the date of the last visit, patient death, or graft loss. Patient survival was calculated from LT to the last visit or patient death. Actuarial survivals were computed with the Kaplan-Meier method, and the differences between groups were compared by the log-rank test. Logistic regression analysis was used with variables that significantly affected SVR in the univariate analysis. A P value < 0.05 was considered statistically significant in all the analyses. Because of the high number of variables analyzed (n = 27), the level of statistical significance was divided by the total number of variables (0.05/27 = 0.002), and only factors reaching this new level of significance were considered in the multivariate analysis.[17] Statistical analysis was carried out with the SPSS software package, version 13.0 (SPSS, Inc., Chicago, IL).

RESULTS

The median follow-up after transplant was 39.2 months (range: 6-95). Twenty-five (25%) patients achieved an

TABLE 1. Univariate Analysis of Factors Predicting Sustained Virological Response After the Treatment of Posttransplant HCV Recurrence (Donor and Intraoperative Variables)

Variable		SVR (n = 25)	*P* Value
Donor gender	Male (n = 65)	16 (25%)	0.8
	Female (n = 34)	9 (26%)	
Donor age	≤60 years (n = 49)	19 (39%)	**0.002**
	>60 years (n = 50)	6 (12%)	
Donor HCV status	Negative (n = 95)	25 (26%)	0.5
	Positive (n = 4)	0 (0%)	
Use of noradrenaline	No (n = 60)	20 (33%)	**0.02**
	Yes (n = 39)	5 (13%)	
Donor AST and ALT > 500 IU/L or bilirubin > 2 mg/mL	No (n = 86)	25 (29%)	0.1
	Yes (n = 7)	0 (0%)	
Donor ICU stay	≤7 days (n = 90)	24 (27%)	0.1
	>7 days (n = 9)	1 (11%)	
Ischemia time	≤8 hours (n = 65)	17 (26%)	0.7
	>8 hours (n = 34)	8 (23%)	
RBC transfusions during LT	≤8 L (n = 93)	23 (25%)	0.6
	>8 L (n = 6)	2 (33%)	

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; ICU, intensive care unit; LT, liver transplantation; RBC, red blood cell.

SVR, whereas 11 (11%) patients had to discontinue AVT because of side effects. Severe HCV recurrence, defined as a fibrosis score ≥ 3[8] and developing before, during, or after AVT, occurred in 42 (42%) patients.

During the study period, there were 29 graft losses (29%). Causes of graft loss were HCV recurrence in 21 cases (21%), infections in 3 cases (3%), recurrence of hepatocellular carcinoma in 2 cases (2%), and other causes in 3 cases (3%).

During the study period, 27 (27%) patients died. Causes of death were HCV recurrence in 17 cases (17%), infections in 4 cases (4%), recurrence of hepatocellular carcinoma in 2 cases (2%), neurological causes in 1 case (1%), and other causes in 3 cases (3%).

Factors Affecting SVR

In the univariate analysis, donor age ≤60 years (P = 0.002), avoidance of noradrenaline in donors (P = 0.02), viral genotype other than 1 (P = 0.002), and use of CyA as the main immunosuppressor (P = 0.001) were all predictors of SVR (Tables 1 and 2).

In the multivariate logistic regression analysis, donor age < 60 years (odds ratio = 4.45, P = 0.012), viral genotype other than 1 (odds ratio = 4.97, P = 0.006), and the use of CyA during treatment (odds ratio = 6.85, P = 0.001) were predictors of SVR. However, only the use of CyA achieved the targeted P value and proved to be an independent predictor of SVR (Table 3).

Comparison Between Patients Receiving CyA or TAC During AVT

Patients receiving CyA as the main immunosuppressant drug during AVT had an SVR rate of 43%, and those treated with TAC had an SVR rate of 14%.

The rate of end-of-treatment biochemical response (defined as normalization of serum alanine aminotransferases at the end of AVT) was 68% (25 patients) in the CyA group and 48% (30 patients) in the TAC group (P = 0.06).

The rate of end-of-treatment virological response (defined as undetectable HCV-RNA at the end of AVT) was 57% (21 patients) in the CyA group and 35% (22 patients) in the TAC group (P = 0.04).

The baseline values of hemoglobin at the initiation of AVT were 12.2 (9.5-16.1) g/dL in the CyA group and 12.5 (9.1-16.8) g/dL in the TAC group (P = 0.6). The baseline values of creatinine were 1.18 (0.73-2.00) mg/dL in the CyA group and 1.20 (0.3-2.10) mg/dL in the TAC group (P = 0.7).

The 2 groups were comparable for all variables considered in the univariate analysis, except for a shorter ischemia time and shorter timing after LT of initiation of AVT in the TAC group (P = 0.02 and P = 0.005, respectively) and a greater use of anti-CD25 antibodies, azathioprine, and MMF in the CyA group (P = 0.03, P < 0.001 and P = 0.001, respectively; Tables 4 and 5).

Three patients in each group were sequentially treated with either IFN or PEG-IFN; in 5 cases, PEG-IFN was introduced because of no response to IFN, whereas in 1 case, AVT was switched from PEG-IFN to IFN because of supposedly better management of side effects. The overall SVR rate in these 6 patients was 0% (Tables 2 and 5). Biochemical and virological responses were obtained in 1 patient (17%) receiving CyA, whereas a severe recurrence was observed in 5 patients (83%). Two (33%) patients immunosuppressed with TAC died because of HCV recurrence 20 and 84 months after LT, respectively.

During AVT, 2 (5%) patients in the Cya group and 1 (2%) patient in the TAC group developed histology-proven rejection episodes, which were successfully

TABLE 2. Univariate Analysis of Factors Predicting SVR After the Treatment of Posttransplant HCV Recurrence (Recipient Variables)

Variable		SVR (n = 25)	*P* Value
Recipient gender	Male (n = 72)	16 (22%)	0.2
	Female (n = 27)	9 (33%)	
Recipient age	≤55 years (n = 47)	11 (23%)	0.6
	>55 years (n = 52)	14 (27%)	
Recipient BMI	≤25 (n = 56)	18 (32%)	0.07
	>25 (n = 43)	7 (16%)	
Viral genotype	1 (n = 65)	10 (15%)	**0.002**
	Other than 1 (n = 34)	15 (44%)	
Timing after LT of AVT	≤6 months (n = 36)	8 (22%)	0.6
	>6 months (n = 63)	17 (27%)	
Pre-AVT HCV-RNA	≤2.4 × 106 IU/mL (n = 48)	13 (27%)	0.6
	>2.4 × 106 IU/mL (n = 51)	12 (23%)	
Pre-AVT AST	≤3 N (n = 47)	11 (23%)	0.6
	>3 N (n = 52)	14 (27%)	
Pre-AVT ALT	≤3 N (n = 35)	5 (14%)	0.06
	>3 N (n = 64)	20 (31%)	
Pre-AVT graft fibrosis	Stages 0-2 (n = 84)	22 (26%)	0.7
	Stages 3-4 (n = 15)	3 (20%)	
Main immunosuppressive drug	Cyclosporine (n = 37)	16 (43%)	**0.001**
	Tacrolimus (n = 62)	9 (14%)	
Post-LT steroid tapering	≤6 months (n = 30)	10 (33%)	0.2
	>6 months (n = 69)	15 (22%)	
Mycophenolate mofetil	No (n = 89)	20 (22%)	0.1
	Yes (n = 10)	5 (50%)	
Rapamycin	No (n = 93)	24 (26%)	1.0
	Yes (n = 6)	1 (17%)	
Anti-CD25 antibodies*	No (n = 89)	20 (22%)	0.1
	Yes (n = 10)	5 (50%)	
Anti-CD52 antibodies†	No (n = 96)	25 (26%)	0.5
	Yes (n = 3)	0 (0%)	
Azathioprine	No (n = 74)	16 (22%)	0.1
	Yes (n = 25)	9 (36%)	
Post-LT CMV infections	No (n = 80)	22 (27%)	0.3
	Yes (n = 19)	3 (16%)	
Post-LT steroid boluses	No (n = 64)	19 (30%)	0.1
	Yes (n = 35)	6 (17%)	
Type of interferon	IFN α-2b (n = 61)	17 (28%)	0.3
	PEG-IFN (n = 32)	8 (25%)	
	IFN α-2b + PEG-IFN (n = 6)	0 (0%)	

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AVT, antiviral treatment; BMI, body mass index; CMV, cytomegalovirus; HCV, hepatitis C virus; IFN, interferon; LT, liver transplantation; PEG-IFN, pegylated interferon; SVR, sustained virological response.
*Including basiliximab and daclizumab.
†Including alemtuzumab.

treated with steroid pulse doses in 2 cases, whereas in 1 case, the patient developed chronic rejection requiring a switch of immunosuppression from CyA to TAC.

The follow-up time was significantly longer in the CyA group versus the TAC group: 52 (7-93) months versus 30 (6-95) months ($P < 0.001$).

Six (16%) patients in the CyA group and 5 (8%) in the TAC group had to discontinue AVT because of side effects ($P = 0.3$). Seven (19%) patients in the CyA group and 14 (23%) in the TAC group lost their graft because of HCV recurrence ($P = 0.6$).

The 5-year patient survival rate after LT was 79% in the CyA group and 68% in the TAC group ($P = 0.2$). The 5-year graft survival rate was 79% in the CyA group and 66% in the TAC group ($P = 0.1$).

DISCUSSION

The present analysis, conducted with a retrospective series of patients transplanted for HCV-related cirrhosis, showed that 3 factors—donor age, viral genotype, and use of CyA as the main immunosuppressor during AVT—were predictors of SVR. In taking into consideration all possible parameters with a potential impact on the course of HCV recurrence, we had to lower the level of significance, and the use of CyA was the only variable

TABLE 3. Multivariate Analysis of Factors Predicting Sustained Virological Response After the Treatment of Posttransplant HCV Recurrence

Variable	*P* Value	Odds Ratio	95% CI
Donor age ≤ 60 years	0.012	4.45	**1.39–14.19**
Viral genotype other than 1	0.006	4.97	**1.59–15.48**
Cyclosporine while on AVT	0.001	6.85	**2.15–21.73**

Abbreviations: AVT, antiviral treatment; CI, confidence interval.

reaching this newly obtained threshold in the multivariate analysis.

The evidence of the superiority of CyA versus TAC in achieving this specific goal could be affected by the retrospective nature of the study and the long time span covered (8 years), but the difference in the SVR rate in the 2 groups was remarkable. The similarity of most of the variables included in the univariate analysis between patients treated with CyA or TAC should in fact minimize the effect of time.

Factors pertaining to graft reinfection (viral genotype, pretreatment levels of HCV-RNA and serum transaminases, and fibrosis stage) were equally represented in the 2 groups. Duration of steroid tapering, use of steroid pulse doses and OKT3, and occurrence of CMV infections—all determinant or presumed factors in accelerating the progression of HCV recurrence[6-11,24]—were also comparable.

Some variables diverged between patients treated with CyA or with TAC. In particular, the timing of starting AVT was shorter in the TAC group. We believe that this difference may be correlated to a more aggressive approach in treating patients with HCV recurrence in recent years, when the utilization of TAC increased, rather than to a slower course of reinfection in patients immunosuppressed with CyA, because of the aforementioned similarity of parameters linked to the aggressiveness of recurrence.

The comparable use of different IFNs in the CyA and TAC groups may be due to the fact that the earlier initiation of AVT in TAC patients was accompanied by the frequent choice of using standard IFN because of the higher possibility of dose modulation in the case of side effects.

The other differences resided in a greater use of anti-CD25 antibodies, MMF, and azathioprine in the Cya group and a greater use of rapamycin in the TAC group. The impact of anti-CD25 antibodies (basiliximab and daclizumab) in post-LT HCV recurrence is still controversial,[25,26] and no data exist on the relationship between anti-CD25 induction therapy and response to AVT.

Even if its usefulness in HCV-positive transplant patients is still debated,[27,28] MMF has been shown to inhibit HCV replication, with a synergistic effect with CyA and IFN α.[29] Thus, although less than one-fourth of patients under CyA simultaneously received MMF, this drug may have enhanced the favorable action of CyA. Conversely, azathioprine was given in combination with CyA in more than 50% of cases, but for the first 6 post-LT months only, as in most common protocols. The role of azathioprine in HCV recurrence is also debated,[27,28] and it is at present difficult to define its effect when the drug was already stopped before AVT was started in the majority of cases. Similarly, the effect of rapamycin on HCV replication remains to be elucidated.

It has to be pointed out, however, that none of these factors with a different prevalence in the 2 groups had a significant impact on SVR.

Our results support the viral suppressive effects of CyA observed in vitro[14] and are in line with previous reports showing that CyA is probably preferable to TAC in patients treated for HCV recurrence.[15-17] However, each of these studies exhibits some differences from ours. Sugawara et al.[15] reported that 63% of living donor transplant recipients who were nonresponders to a pre-emptive AVT eventually displayed SVR after conversion from TAC to Cya and maintenance of AVT.

Bizollon et al.[17] examined the SVR rate of nonresponders to previous AVT with nonpegylated IFN and ribavirin after retreatment with PEG-IFN, demonstrating better results in comparison with a group of untreated patients during the same period. In their analysis, CyA during retreatment was significantly associated with viral clearance.

The study by Firpi et al.[16] is probably most similar to ours; they obtained an SVR rate of 46% in CyA-treated patients versus 27% in TAC-treated patients with post-LT HCV recurrence receiving AVT. However, the setting was different because the median donor age was 20 years younger than that of our population. Older donor age may have represented an adverse factor and partially accounted for the very low rate of SVR in patients treated with TAC in our study.

The number of subjects considered in our series was higher than that of 2 of the cited reports and comparable to that analyzed in the third one. In addition, the number of variables that we investigated was much higher than that of any other study; this may be disproportional to the number of patients but also necessary to reliably explore the real predictors of SVR.

In the present analysis, the overall incidence of severe recurrence and graft loss due to HCV recurrence was high. An improvement of fibrosis in patients treated with PEG-IFN has been reported, regardless of the achievement of SVR.[17] We did not aim to assess histological changes after AVT; however, patients treated with CyA had better (though not significantly) survival rates and a much longer follow-up than those treated with TAC, and this may be a prelude to significantly worse results in this group in the long term.

In fact, the unsolved dispute about which immunosuppressive drug is preferable in HCV-positive subjects is usually based on the analysis of outcomes of all HCV-positive patients undergoing LT,[13] where many variables may play a role, including the prevalence of

TABLE 4. Donor and Operative Variables of Patients Treated for HCV Recurrence According to the Main Immunosuppressant Used at the Initiation of Treatment

Variable	Cyclosporine (n = 37)	Tacrolimus (n = 62)	*P* Value
Donor gender (male/female)	22/15	43/19	0.3
Donor age > 60 years	18 (49%)	32 (52%)	0.7
Positive donor HCV status	2 (5%)	2 (3%)	0.5
Use of noradrenaline	12 (32%)	27 (43%)	0.2
Donor AST and ALT > 500 IU/L or bilirubin > 2 mg/mL	2 (6%)	5 (9%)	0.7
Donor macrovesicular steatosis > 30%	1 (3%)	1 (2%)	1.0
Donor ICU stay > 7 days	3 (8%)	6 (10%)	1.0
Ischemia time > 8 hours	18 (49%)	16 (26%)	**0.02**
RBC transfusions during LT > 8 L	3 (8%)	3 (5%)	0.6

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; ICU, intensive care unit; LT, liver transplantation; RBC, red blood cell.

TABLE 5. Demographic, Clinical, and Pathological Parameters of Patients Treated for HCV Recurrence According to the Main Immunosuppressant Used at the Initiation of Treatment

Variable	Cyclosporine (n = 37)	Tacrolimus (n = 62)	*P* Value
Recipient gender (male/female)	24/13	48/14	0.1
Recipient age > 55 years	19 (51%)	33 (53%)	0.8
Recipient BMI > 25	13 (35%)	30 (48%)	0.1
Viral genotype 1	25 (68%)	40 (64%)	0.7
Timing from LT of AVT ≤ 6 months	7 (19%)	29 (47%)	**0.005**
Pre-AVT HCV-RNA > 2.4×10^6 IU/mL	21 (57%)	30 (48%)	0.4
Pre-AVT AST > 3 N	21 (57%)	31 (50%)	0.5
Pre-AVT ALT > 3 N	25 (68%)	39 (63%)	0.6
Pre-AVT graft fibrosis > 2	7 (19%)	8 (13%)	0.4
Post-LT steroid tapering ≤ 6 months	13 (48%)	17 (33%)	0.1
Mycophenolate mofetil	9 (24%)	1 (2%)	**0.001**
Rapamycin	0 (0%)	6 (10%)	0.08
Anti-CD25 antibodies*	7 (19%)	3 (5%)	**0.03**
Anti-CD52 antibodies†	0 (0%)	3 (5%)	0.3
Azathioprine	21 (57%)	4 (6%)	<**0.001**
Post-LT CMV infections	9 (24%)	10 (16%)	0.3
Post-LT steroid pulse doses	11 (30%)	24 (39%)	0.3
Post-LT OKT3	1 (3%)	1 (2%)	1.0
Type of interferon			0.7
IFN α-2b	22 (59%)	39 (63%)	
PEG-IFN	12 (32%)	20 (32%)	
IFN α-2b + PEG-IFN	3 (8%)	3 (5%)	

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AVT, antiviral treatment; BMI, body mass index; CMV, cytomegalovirus; HCV, hepatitis C virus; IFN, interferon; LT, liver transplantation; PEG-IFN, pegylated interferon.
*Including basiliximab and daclizumab.
†Including alemtuzumab.

patients receiving AVT and all factors related to its application. The selective evaluation of patients treated for HCV recurrence is probably a more rational and useful approach.

In summary, this retrospective study has shown that donor age, viral genotype, and use of CyA are the most important predictors of SVR in patients treated for HCV recurrence after LT. On the basis of the obtained results, a prospective trial in which patients with histological recurrence are enrolled in a program of AVT with PEG-IFN and ribavirin and are randomly assigned to receive CyA or TAC is needed.

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