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ORIGINAL ARTICLE

Early Viral Load and Recipient Interleukin-28B rs12979860 Genotype Are Predictors of the Progression of Hepatitis C After Liver Transplantation

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There have been few detailed studies of viral kinetics after liver transplantation (LT), and conflicting data have been reported on viral loads and the severity of recurrent hepatitis C virus (HCV) disease. This long-term study aimed to examine (1) the impact of HCV RNA levels at specific points in time within the first year and (2) the influence of interleukin-28B (IL-28B) genotypes on patient outcomes and the severity of recurrent HCV disease. The viral loads were measured 2, 4, 12, 24, and 48 weeks after LT, and the recipient/donor IL-28B genotypes of 164 patients were determined. A Cox regression analysis showed that the viral load at week 2 was an independent negative predictor of recipient outcomes. A week 2 viral load $\geq$ 6.0 $\log_{10}$ IU/mL was significantly associated with reduced patient survival. After a mean follow-up of 6.5 years, 21 of 164 patients (12.8%) developed a cholestatic type of HCV recurrence and/or rapidly progressed to cirrhosis within 1 year. A multivariate binary regression analysis showed that HCV viremia at week 2 and a non-C/C recipient IL-28B genotype were independent risk factors for cholestatic recurrent HCV. No predictive factors could be found for the occurrence of recurrent liver cirrhosis 5 and 10 years after LT. Our study shows that the HCV RNA level at week 2 and the recipient IL-28B genotype are independent, statistically significant risk factors for post-LT cholestatic HCV, and it emphasizes the importance of viral load monitoring and IL-28B genotyping for identifying HCV recipients at risk for severe HCV recurrence. ***Liver Transpl 18:671-679, 2012.*** © **2012 AASLD.**

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Hepatitis C virus (HCV)–related cirrhosis is one of the most common indications for liver transplantation (LT) and accounts for approximately 40% of all LT procedures. Reinfection of the liver allograft is virtually universal and occurs during reperfusion.[1,2] Histological recurrence varies widely from patient to patient. The majority of HCV patients have minimal or nonprogressive liver injury after LT and good long-term survival. In a subgroup of patients, however, a dramatic course of reinfection is associated with poor outcomes and is characterized by the development of either liver cirrhosis or cholestatic hepatitis.[1–4] Cirrhosis due to recurrent HCV occurs in at least 25% of patients within 5 to 10 years after LT, and once cirrhosis has developed, there is an annual risk of decompensation of 42%.[2,3,5,6] Recurrent cholestatic hepatitis is an infrequent but extremely severe manifestation of HCV recurrence that leads to graft failure within a few months of its onset in the majority of patients. As a result, several studies have reported that the long-term outcomes

Abbreviations: bDNA, branched DNA; CNI, calcineurin inhibitor; CYA, cyclosporine A; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IL-28B, interleukin-28B; LT, liver transplantation; PCR, polymerase chain reaction; TAC, tacrolimus.

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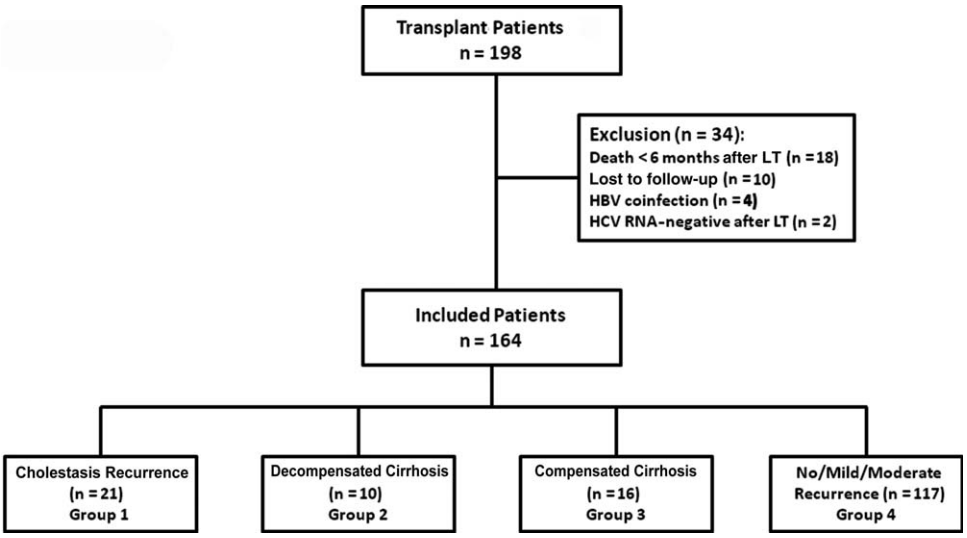

Figure 1. Flow diagram for all recipients with HCV.

of HCV patients are poorer than the outcomes of patients with non–HCV-related diseases.[4,7,8]

Donor age, donor steatosis, and acute rejection episodes treated with steroid bolus therapy and/or monoclonal antibodies are generally accepted to be parameters associated with a negative impact on the severity of recurrent HCV.[9,10]

So far, there have been relatively few studies of detailed viral kinetics after LT with relevance to long-term outcomes. HCV levels have been shown to decrease significantly after hepatectomy and during the anhepatic phase and to continue to decline within the first 12 to 24 hours after LT, but they increase rapidly from week 2, and HCV RNA levels usually peak around month 4.[11–14] The relationships between the viral load and both the severity of HCV recurrence and long-term patient outcomes after LT have been contradictory.[14–19] A more recent article emphasized the importance of a high peak viral load $> 10^7$ IU/mL within the first year after LT as an independent predictor of poor recipient outcomes.[13]

Recent publications have provided strong evidence for the association of single-nucleotide polymorphisms on chromosome 19 near the interleukin-28B (IL-28B) gene with treatment-related and spontaneous clearance of HCV infection.[20,21] So far, few data have been available about the impact of the IL-28B genotype on the response to antiviral therapy, and only preliminary results have been published about IL-28B polymorphisms and the natural course of HCV reinfection in the LT setting.[22–26]

In this study, we aimed to determine whether post-transplant HCV RNA kinetics within the first year after LT could be used to predict the long-term outcomes of HCV patients and the severity of recurrent disease. We retrospectively analyzed the relationships of several prospectively collected parameters, including HCV RNA levels at various points in time within the first year and recipient and donor IL-28B genotypes, with the development of cholestatic hepatitis and/or progressive fibrosis in order to identify HCV recipients with the highest risk for poor long-term outcomes as early as possible.

PATIENTS AND METHODS

Study Design

In this single-center study, we retrospectively analyzed prospectively collected data.

This study was approved by the local ethics committee.

Patient Selection (Fig. 1)

Between 1994 and 2009, 198 patients underwent transplantation for HCV cirrhosis at our institution. All patients were included in the overall survival analysis. However, only patients who survived more than 6 months with a histological assessment of the absence or presence of recurrent HCV were included in this study to address the impact of viral loads on HCV patient outcomes after LT. Thirty-four patients (17.2%) were excluded: 18 died within 6 months after LT because of infectious complications (none because of recurrent HCV), and 10 patients were lost to follow-up or had no available liver biopsy or viral load measurements. Four patients with a hepatitis B virus (HBV) coinfection and 2 patients with negative HCV RNA findings after LT were also excluded.

In all, 164 patients fulfilled our inclusion criteria and composed our study group. For statistical analysis, the patients were divided into 4 groups. In group 1, we included patients who developed severe recurrent disease and presented with a cholestatic type of recurrent hepatitis and/or rapidly progressed to an advanced fibrosis stage (F3-F4) within 1 year after LT. The diagnosis of post-LT cholestatic HCV was based

TABLE 1. Clinical Characteristics of Patients

	Overall Group (n = 198)	Study Group (n = 164)
Sex: female/male (n/n)	44/154	33/131
Age at LT (years)*	56.3 ± 9.1 (21.7-73.2)	56.4 ± 8.8 (36.4-73.2)
Genotype [n (%)]		
1	125 (63.1)	106 (64.6)
2	19 (9.6)	16 (9.7)
3	25 (12.7)	26 (15.9)
4	6 (3.0)	8 (4.9)
Not available	23 (11.6)	8 (4.9)
Concomitant HCC [n (%)]	90 (45.5)	70 (42.7)
Donor age (years)*	38.1 ± 16.0 (14-76)	38.4 ± 16.6 (18-73)
Follow-up (years)*	6.5 ± 4.7 (0.1-17.4)	7.1 ± 4.6 (0.6-17.4)
Retransplantation [n (%)]	16 (8.1)	11 (6.7)
HCV recurrence (n)	5	5
Primary nonfunction (n)	7	3
Secondary biliary cirrhosis due to nonanastomotic biliary strictures (n)	3	2
Ductopenic rejection (n)	1	1

*Data are presented as means and standard deviations with ranges in parentheses.

on the definition published by the International Liver Transplantation Society in 2003.[27] Patients who presented with liver cirrhosis after the first post-LT year were separated according to the presentation of decompensation (group 2 included patients with decompensated cirrhosis, and group 3 included patients with compensated cirrhosis). Patients with absent or mild to moderate (F0-F2) disease were included in group 4. The mean follow-up of all patients was 6.5 ± 4.7 years (median = 5.9 years, range = 0.1-17.4 years).

Hepatocellular carcinoma (HCC) was diagnosed in 70 study patients either before LT or in the explanted liver. The majority of the study patients presented with HCV genotype 1 (64.6%); this was followed by genotype 2 (9.7%), genotype 3 (15.9%), and genotype 4 (4.9%). For 4.9% of the patients, the genotype was not available. As summarized in Table 1, the main characteristics of the patients did not differ between the overall group and the study group.

Histological Analysis

Before 1997, liver biopsy was performed only for patients with elevated serum transaminases. Thereafter, specimens for liver histology were obtained prospectively according to a fixed protocol, regardless of the biochemical profile. The first liver biopsy was performed 1 year after LT or earlier if it was clinically indicated to differentiate between recurrent HCV and rejection. Afterward, liver biopsy was performed on an annual basis.

The modified hepatitis activity index and the fibrosis score were determined for all biopsy specimens.[28] Liver biopsy samples were interpreted by experienced liver pathologists who were blinded to the clinical and virological data.

Virological Data

The diagnosis of HCV was based on the detection of anti-HCV and HCV RNA in the serum by polymerase chain reaction (PCR) before and after LT. Between 1994 and 2000, the viral loads were measured from stored sera (−80°C) prospectively collected during the corresponding follow-up visits (week 2 and months 1, 3, 6, and 12) with a branched DNA (bDNA) assay (version 2) from Chiron. Between 2000 and 2003, an HCV monitoring test from Roche (linearity = 100-500,000 IU/mL) was used. In 2004, we began to measure the viral loads with the HCV RNA 3.0 assay from Bayer (linearity = 615-7700,000 IU/mL). The vast majority of the samples were measured with the HCV RNA 3.0 test system. All values were converted ($\log_{10}$ IU/mL). Previous studies have shown good correlations between the bDNA 3.0 assay, the second-generation bDNA assay, and the PCR-based Cobas Amplicor HCV Monitor test.[29,30]

The HCV genotype was assessed with the INNO-LiPA test (Innogenetics, Brussels, Belgium).

IL-28B Genotyping

DNA was extracted either from peripheral blood monocytes with the QIAamp DNA blood mini kit or from paraffin-fixed liver tissue blocks from the explanted liver with the PAXgene tissue DNA kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). Tissue was used if whole blood was not available (n = 14). Recipient DNA and donor DNA were genotyped for the DNA polymorphism rs12979860 near the IL-28B gene with a custom

TaqMan single-nucleotide polymorphism genotyping assay (ABI, Carlsbad, CA) according to the manufacturer's instructions. Briefly, the reaction was performed with 0.5 μg of genomic DNA, which was brought to a final volume of 12.5 μL with primers, probes, and 6.25 μL of the TaqMan universal PCR master mix. The reaction was performed with a StepOne real-time PCR system (ABI, Carlsbad, CA), and the raw data were analyzed with StepOne 2.2.1 (ABI).

Immunosuppression

The immunosuppressive therapy consisted of a triple-drug regimen of cyclosporine A (CYA; 94 patients) or tacrolimus (TAC; 70 patients), corticosteroids, and either azathioprine or mycophenolate mofetil for at least 1 year. Corticosteroids were gradually tapered and were discontinued within 3 months. After 2002, all HCV patients received a daily corticosteroid maintenance dose of 5 mg for at least 1 year in an attempt to slow fibrosis progression, as suggested by Brillanti et al.[31] The mammalian target of rapamycin inhibitors sirolimus and everolimus were used in 25 patients.

Statistical Analysis

The Kaplan-Meier method was used to estimate overall survival. The log-rank test was performed to evaluate survival differences between specific groups of patients. A Cox regression model was used to analyze the independent effects of different variables on survival. Binary logistic regression was used to analyze different variables associated with the development of recurrent cholestatic hepatitis and/or rapid progression to liver cirrhosis. Because of the imbalance between patients with or without cholestatic recurrent disease and progression to cirrhosis, these patients were weighted by a factor of 5 or higher. In all these statistical models, we found the same results. A P value ≤ 0.05 was considered statistically significant.

The calculations were performed with SPSS 15.0 for Windows (SPSS Co., Chicago, IL).

RESULTS

Type of HCV Recurrence

Twenty of the 164 patients (12.2%) developed a cholestatic type of HCV recurrence, and 1 patient rapidly progressed to cirrhosis within 1 year of LT without a cholestatic laboratory profile (group 1). Ten of the 20 patients with a cholestatic type of recurrence (50%) subsequently progressed to liver cirrhosis but remained in the cholestatic group for statistical analysis. In 26 patients (15.9%), cirrhosis of the allograft was diagnosed at a mean of 5.3 ± 3.0 years (median = 4.8 years, range = 1.4-13.2 years) after transplantation; 10 of these patients (6.1%) developed decompensated liver disease (group 2), and 16 (9.8%) did not (group 3). The remaining 117 patients showed either no evidence of recurrence or only mild to moderate HCV recurrence (group 4; Fig. 1).

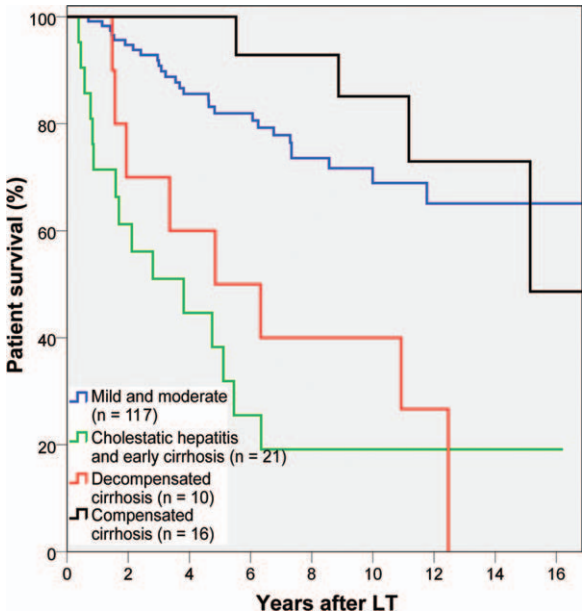

Figure 2. Patient survival for the overall study cohort (n = 164) according to the type of HCV recurrence ($P < 0.001$).

Overall Survival

The actuarial patient survival rates of all 198 HCV patients who underwent LT at our center were 88%, 73%, and 62% at 1, 5, and 10 years, respectively. These rates were comparable to the rates for other indications at our institution.

The overall patient survival rates of our study group (n = 164) were 96%, 90%, 77%, and 63% at 1, 2, 5, and 10 years, respectively. Patients in group 1 had significantly decreased survival rates at 1, 5, and 10 years (71%, 39%, and 20%, respectively) in comparison with patients in group 4 (99%, 83%, and 70%, respectively; $P < 0.001$). Interestingly, the survival of patients with compensated cirrhosis (group 3) was similar to the survival of patients in group 4. However, the survival of patients with decompensated cirrhosis significantly dropped: the 1-, 5- and 10-year survival rates were 100%, 50%, and 40%, respectively (Fig. 2).

Fifteen of the 20 patients (75%) with cholestatic disease died; all deaths were due to liver failure associated with sepsis and multiorgan failure. Seven of the 10 patients with decompensated cirrhosis died [liver failure (6) or coronary heart disease (1)]. Two patients underwent re-LT, and 1 patient was on the waiting list for re-LT.

A Cox regression analysis showed that only the viral load at week 2 was an independent predictor of reduced patient survival. In particular, a viral load at week 2 ≥ 6.0 $\log_{10}$ IU/mL was associated with poor outcomes ($P = 0.006$; Fig. 3). Within a median period of 13.3 months (range = 6.9-106 months) after LT, 16 of 37 patients (43%) with a viral load ≥ 6.0 $\log_{10}$ IU/mL died; 10 of these deaths were due to recurrent HCV [cholestatic hepatitis (7) or decompensated cirrhosis (3)]. Six patients died because of non–HCV-related causes (coronary heart disease, HCC

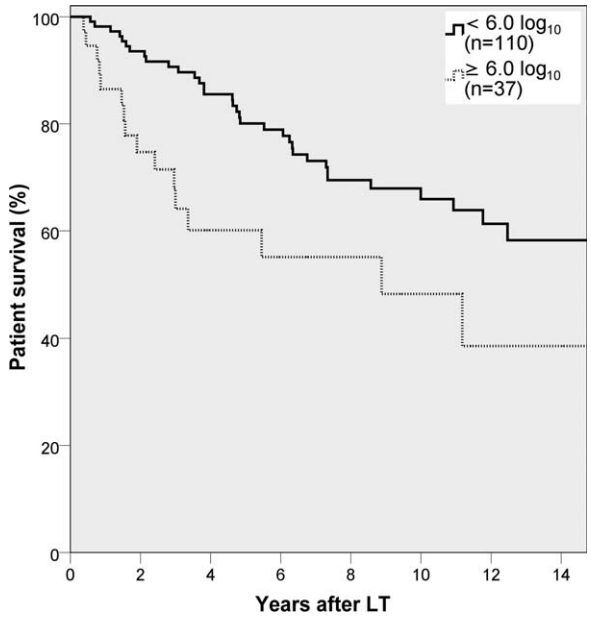

Figure 3. Effect of HCV RNA levels at week 2 on patient survival. A Kaplan-Meier plot shows patient survival according to week 2 HCV viral loads (<6.0 or ≥6.0 $\log_{10}$ IU/mL) regardless of the type of recurrence ($P = 0.006$).

recurrence, or de novo malignancy). Histological assessments were available for 3 of these patients and showed recurrent HCV (F1-F2). Twenty-one patients with an HCV RNA level ≥ 6.0 $\log_{10}$ IU/mL survived. Ten of these patients, however, developed liver cirrhosis, and 11 patients (29.7%) had necroinflammatory activity and/or a fibrosis score as high as 2 at the last histological assessment.

Other parameters such as the donor age, the cold ischemia time, the genotype, the viral load (months 1, 3, 6, and 12), and the recipient and donor IL-28B genotypes were not statistically significant predictors of survival (Table 2).

TABLE 2. Univariate Cox Regression Analysis: Patient Survival

	Hazard Ratio (95% Confidential Interval)	*P* Value
Genotype	1.06 (0.78-1.45)	0.71
Viral load		
Week 2	**1.51 (1.16-1.98)**	**0.003**
Month 1	1.22 (0.92-1.62)	0.17
Month 3	1.16 (0.86-1.56)	0.33
Month 6	1.03 (0.74-1.43)	0.85
Month 12	0.98 (0.70-1.35)	0.88
Viral load (week 2)		
≥6.0 $\log_{10}$ IU/mL	2.27 (1.25-4.13)	0.007
≥6.5 $\log_{10}$ IU/mL	2.31 (1.12-4.78)	0.02
Recipient age (years)	1.02 (0.99-1.05)	0.20
Recipient sex	0.93 (0.49-1.76)	0.82
IL-28B polymorphism	0.97 (0.56-1.70)	0.92
Donor age (years)	1.00 (0.99-1.02)	0.68
Donor sex	1.52 (0.76-3.04)	0.23
Year of LT	0.86 (0.60-1.21)	0.38
Cold ischemia time (hours)	1.00 (0.99-1.00)	0.88
Anhepatic phase	1.00 (0.99-1.02)	0.59
Post-LT antiviral therapy	0.87 (0.43-1.78)	0.70
Primary CNI (CYA versus TAC)	0.99 (0.57-1.73)	0.98

Graft Survival

In all, 11 patients underwent retransplantation (12 procedures in all); 1 patient underwent retransplantation twice. Five patients underwent retransplantation because of cholestatic hepatitis (n = 1) or decompensated allograft cirrhosis (n = 4). The patient with cholestatic recurrence died shortly after re-LT because of sepsis and subsequent multiorgan failure. Three patients with decompensated cirrhosis of the first allograft survived with no evidence of severe recurrent HCV disease. One patient died because of metastatic prostate cancer. The causes for all re-LT procedures are listed in Table 1.

Viral Loads

The viral loads were measured at week 2 and months 1, 3, 6, and 12. The mean viral loads of all patients gradually increased from week 2 to month 6 and remained stable until month 12: 5.25 ± 1.11 $\log_{10}$ IU/mL at week 2, 5.72 ± 1.08 $\log_{10}$ IU/mL at month 1, 6.06 ± 1.03 $\log_{10}$ IU/mL at month 3, 6.24 ± 0.91 $\log_{10}$ IU/mL at month 6, and 6.15 ± 0.87 $\log_{10}$ IU/mL at month 12.

Patients with a severe recurrent HCV infection (group 1) had higher viral loads at all points in time; however, the difference was significant only during earlier time periods: week 2 ($P = 0.006$), month 1 ($P = 0.006$), and month 3 ($P = 0.05$). In contrast, the mean viral loads of patients with decompensated or compensated cirrhosis were not significantly higher at any points in time than the viral loads of patients with absent or mild/moderate recurrent disease. The viral load kinetics of all 4 groups are depicted in Table 3.

We could not find differences in HCV RNA levels between specific immunosuppression regimens (CYA versus TAC or use versus nonuse of mammalian target of rapamycin inhibitors).

IL-28B Genotype Frequencies

DNA from 151 LT recipients (92%) was successfully typed for the IL-28B polymorphism rs12979860. The C/C and non-C genotype frequencies were in a Hardy-Weinberg equilibrium. The C/C, C/T, and T/T genotypes were found in 24.5%, 61.6%, and 13.9% of patients, respectively. In comparison with patients with no or mild/moderate recurrent disease, patients who developed recurrent cholestatic hepatitis had a significantly lower frequency of the recipient C/C genotype (Table 4). No differences in IL-28B genotype frequencies were seen between patients with

TABLE 3. Viral Load Kinetics

	Week 2	Month 1	Month 3	Month 6	Month 12
Group 1 (log_{10} IU/mL)	5.93 ± 0.94	6.24 ± 0.91	6.54 ± 0.99	6.62 ± 0.94	6.38 ± 1.24
Group 2 (log_{10} IU/mL)	5.18 ± 0.97	5.36 ± 1.36	6.03 ± 0.75	6.36 ± 0.73	6.17 ± 0.95
Group 3 (log_{10} IU/mL)	5.44 ± 1.03	6.01 ± 1.05	6.52 ± 0.75	6.59 ± 0.41	6.59 ± 0.49
Group 4 (log_{10} IU/mL)	5.11 ± 1.12	5.61 ± 1.07	5.91 ± 1.05	6.12 ± 0.95	6.06 ± 0.84
P value	0.02	0.04	0.01	0.08	0.11

NOTE: Data are presented as means and standard deviations.

TABLE 4. Frequencies of the Recipient IL-28B Genotypes According to the Type of Recurrent Disease

Genotype	Cholestatic Hepatitis (%)	Decompensated Cirrhosis (%)	Compensated Cirrhosis (%)	No/Mild/Moderate Recurrence (%)
C/C*	5	63	13	26
C/T	70	27	80	60
T/T	25	0	7	14

NOTE: The recipient IL-28B genotype was available for 151 of the 164 patients (92%).
*For the C/C genotype, *P* was less than 0.05 (chi-square test) between cholestatic hepatitis and no/mild/moderate recurrence.

TABLE 5. Frequencies of the Donor IL-28B Genotypes According to the Type of Recurrent Disease

Genotype	Cholestatic Hepatitis (%)	Decompensated Cirrhosis (%)	Compensated Cirrhosis (%)	No/Mild/Moderate Recurrence (%)
C/C	50	50	33	41
C/T	50	50	67	44
T/T	0	0	0	15

NOTE: The donor IL-28B genotype was available for 53 of the 164 patients (32%). *P* was not significant (chi-square test) between groups.

compensated or decompensated cirrhosis and patients with mild recurrence (Table 4).

Donor DNA was available for 32% of the cases, and IL-28B genotyping was successful for all these donor samples. The C/C, C/T, and T/T genotypes were seen in 43.4%, 45.3%, and 11.3%, respectively. No association could be found between the donor IL-28B genotype and the development of recurrent cholestatic HCV and/or progression to liver cirrhosis (Table 5). We also saw no association between combined IL-28B genotypes and the type of recurrence.

No correlation could be found between the IL-28B genotype and the viral loads at any points in time.

Risk Factors for HCV Recurrence

Cholestatic Recurrent Hepatitis (Table 6)

In a univariate binary logistic regression analysis, the donor age, the viral loads at week 2 and month 1, and the recipient IL-28B genotype (C/C versus C/T and T/T) were found to be predictive factors for the development of cholestatic hepatitis. In a multivariate analysis, viremia at week 2 and the recipient IL-28B genotype (non-C/C) remained independent risk factors for cholestatic HCV recurrence. Week 2 viral loads $\geq$ 6.0 log_{10} IU/mL and $\geq$ 6.5 log_{10} IU/mL were found in 50% and 39% of the patients with cholestatic hepatitis and in only 20% and 9% of the patients with no or mild recurrent disease ($P = 0.01$). Patients with a week 2 viral load $\geq$ 6.0 log_{10} IU/mL had a 3.6-fold increased risk of developing cholestatic recurrent HCV, and patients with a viral load $\geq$ 6.5 log_{10} IU/mL at week 2 had a 4.5-fold increased risk.

Recurrent Decompensated Cirrhosis (Table 7)

None of the aforementioned parameters were significant predictors of decompensated allograft cirrhosis.

Antiviral Therapy After LT

In all, 23 patients (14%) were treated with antiviral therapy after LT. Cholestatic liver disease was the

TABLE 6. Cholestatic Disease: Univariate and Multivariate Binary Logistic Regression Analyses

Univariate Factor	*P* Value
Viral load	
Week 2	**0.008**
Month 1	0.02
Month 3	0.06
Month 6	0.28
Month 12	0.13
Genotype	0.30
Viral load (week 2)	
$\geq$6.0 $\log_{10}$ IU/mL	0.01
$\geq$6.5 $\log_{10}$ IU/mL	0.01
Recipient age (years)	0.34
Recipient sex	0.49
Recipient IL-28B polymorphism	**0.02**
Donor age (years)	**0.01**
Donor sex	0.49
Year of LT	0.68
Cold ischemia time (hours)	0.61
Anhepatic phase	0.27
Primary CNI (CYA versus TAC)	0.48

Multivariate Factor	Hazard Ratio (95% Confidence Interval)	*P* Value
Viral load (week 2)	**2.18 (1.26-3.79)**	**0.006**
IL-28B polymorphism	**2.92 (1.15-7.42)**	**0.025**

TABLE 7. Decompensated Cirrhosis: Univariate Binary Logistic Regression Analysis

Factor	*P* Value
Viral load	
Week 2	0.46
Month 1	0.18
Month 3	0.28
Month 6	0.13
Month 12	0.07
Genotype	0.13
Recipient age (years)	0.41
Recipient sex	0.52
IL-28B polymorphism	0.53
Donor age (years)	0.72
Donor sex	0.98
Year of LT (<1995>)	0.40
Cold ischemia time (hours)	0.82
Anhepatic phase	0.63
Primary CNI (CYA versus TAC)	0.15
Post-LT antiviral therapy	0.46
Response to antiviral therapy	0.12

reason for treatment in 7 patients; rapid progression to liver cirrhosis was the reason for 9 patients. The remaining patients were treated within a randomized, multicenter study.[32] The sustained virological response rate was 48% (39% for patients with genotype 1 or 4 and 80% for patients with genotype 2 or 3). SVR was achieved by 1 of the 7 patients with cholestatic HCV recurrence, and the patient's graft function improved dramatically. The most recent biopsy sample showed no evidence of necroinflammatory activity and only mild fibrosis. The recipient and donor IL-28B genotypes and the different immunosuppression regimens did not show a statistically significant impact on the response to antiviral therapy.

DISCUSSION

In this study, we were able to show that the viral loads within the first postoperative month and the recipient IL-28B genotypes were strong predictive factors for the severity of HCV recurrence and for the long-term outcomes of HCV recipients after LT. A Cox regression analysis showed that the viral load at week 2 was an independent negative predictive factor for patient outcomes. Our study is the first to show that both the level of viremia and, more importantly, the viral load at a specific point in time during the posttransplant course are associated with an increased risk of developing severe recurrent HCV disease and, consequently, with poor patient outcomes after LT. Recently, Shackel et al.[13] reported that a peak viral load greater than 10^7 IU/mL within the first year was an independent predictor of reduced patient and graft survival and HCV-related allograft failure, but in contrast to our study, no further analysis of the association between HCV RNA levels at specific points in time and poor outcomes was provided.

Cholestatic hepatitis is an infrequent but extremely severe type of recurrent disease leading to graft failure within a few months of its onset in more than 50% of patients.[9] Our study has confirmed the devastating prognosis of this entity with a mortality rate of 75%. In the multivariate analysis, the recipient IL-28B genotype (non-C/C) and the viral load at week 2 were independent risk factors for the development of cholestatic hepatitis. HCV RNA levels at week 2 that were greater than or equal to 6.0 and 6.5 $\log_{10}$ IU/mL were associated with 3.6- and 4.5-fold increased risks, respectively, for cholestatic hepatitis and, consequently, a poor prognosis after LT. A similar observation was reported by Doughty et al.,[33] who showed in a small cohort of HCV recipients that cholestatic hepatitis after LT was associated with persistently high serum HCV viral loads within the first postoperative year.

To our knowledge, this is the first study demonstrating an association between the recipient IL-28B genotype and cholestatic recurrent HCV. Patients with a non-C/C recipient genotype had an almost 3-fold increased risk of developing a severe recurrence of HCV. There is increasing evidence that the IL-28B polymorphism is a strong predictor of a sustained virological response in patients treated for recurrent HCV after LT.[23–26] Although 1 study showed equal effects of donor and recipient IL-28B genotypes on the virological response,[23] another publication demonstrated a more dominant impact of the donor IL-28B

genotype on the treatment outcome.[24] Very few available data suggest that the IL-28B polymorphism may determine the severity of the histological recurrence of HCV.[23,24,34,35] One recent study reported that the recipient IL-28B genotype (but not the donor IL-28B genotype) was significantly predictive of the fibrosis stage, with the T/T genotype being associated with more rapid fibrosis.[23] Our data confirm that the recipient IL-28B polymorphism (rather than the donor IL-28B polymorphism) influences the severity of HCV recurrence. The small donor DNA sample size, however, might limit our observations about the donor IL-28B genotype and the development of cholestatic recurrent HCV. In contrast, a recent publication could not find an association between the donor and recipient IL-28B genotypes and the occurrence of liver allograft cirrhosis or the time to decompensation.[24] In accordance with previous reports,[23,24] the recipient and donor IL-28B genotypes did not show any impact on graft or patient survival.

So far, we can only speculate about the mechanism underlying the association between the recipient IL-28B polymorphism and the severity of HCV recurrence. Charlton et al.[23] suggested that the recipient IL-28B genotype may influence fibrosis progression by regulating the HCV-specific, human leukocyte antigen–independent adaptive immune response through the activation of dendritic cells, T lymphocytes, or plasma cells.[23] The pathogenesis of cholestatic HCV after LT is also poorly understood.[4,36] It has been proposed that a direct cytopathic effect of HCV causes hepatocellular injury in the presence of high viral loads in the early postoperative period (as confirmed by our study) and a large number of HCV virions in hepatocytes.[4,36] Furthermore, a predominant T helper 2 immune response and quasispecies have been implicated in the pathogenesis of cholestatic recurrent HCV.[37,38] In this study, we were able to demonstrate an association between the recipient IL-28B genotype and cholestatic hepatitis after LT, and we suggest that genetic polymorphisms in recipient-driven extrahepatic tissues and/or liver-infiltrating innate or adaptive immune cells may be involved in the development of cholestatic HCV recurrence. However, further studies are needed to understand the exact role of IL-28B polymorphisms in the postoperative course of HCV recipients.

Many studies have shown that advanced donor age is associated with poor patient survival.[9,13,39,40] In our study, donor age was an independent predictor in the univariate analysis, but it did not reach statistical significance in the multivariate analysis. Our findings suggest that patients who receive allografts from older donors, present with HCV RNA levels $\geq$ 6.0 $\log_{10}$ IU/mL at week 2, and possess a non-C/C recipient IL-28B genotype have a significantly increased risk of developing cholestatic recurrent hepatitis and having a poor outcome after LT. Our data suggest that preemptive antiviral therapy should be considered for these high-risk patients to prevent severe disease recurrence and to improve patient survival. The poor responses of patients with a non-C/C IL-28B genotype support the urgent search for better treatment options with direct-acting antivirals also in the post-transplant setting. These findings call for prospective trials to study the efficacy of early antiviral therapy with direct-acting antivirals in patients with the highest risk of severe recurrent disease as determined by high viral loads within the first month of the post-transplant course and a non-C/C recipient IL-28B genotype.

In conclusion, our data emphasize the importance of regular viral load monitoring after LT and the idea that the recipient IL-28B genotype should be taken into account in order to identify HCV recipients at risk for severe cholestatic HCV recurrence.

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