TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Treatment of genotype-1 hepatitis C recurrence after liver transplant improves survival in both sustained responders and relapsers

Francesca Romana Ponziani,[1] Alessandro Milani,[1] Antonio Gasbarrini,[1] Raffaella Zaccaria,[1] Raffaella Viganò,[2] Rosa Maria Iemmolo,[3] Maria Francesca Donato,[4] Maria Rendina,[5] Pierluigi Toniutto,[6] Luisa Pasulo,[7] Matteo Cescon,[8] Patrizia Burra,[9] Lucia Miglioresi,[10] Manuela Merli,[11] Daniele Di Paolo,[12] Stefano Fagiuoli,[7] and Maurizio Pompili[1] on behalf of AISF RECOLT-C Group

1 Agostino Gemelli Hospital, Rome, Italy
2 Niguarda Ca' Granda Hospital, Milan, Italy
3 University of Modena, Modena, Italy
4 IRCCS Foundation Ca' Granda Maggiore Hospital, Milan, Italy
5 University of Bari, Bari, Italy
6 University of Udine, Udine, Italy
7 Ospedali Riuniti, Bergamo, Italy
8 Sant'Orsola Malpighi Hospital, Bologna, Italy
9 University of Padua, Padua, Italy
10 San Camillo Spallanzani Hospital, Rome, Italy
11 Sapienza University, Rome, Italy
12 University of Torvergata, Rome, Italy

Keywords
antiviral treatment, genotype-1, hepatitis C recurrence, hepatitis C virus, liver transplant, pegylated interferon, ribavirin, responders, survival.

Correspondence
Dr. Francesca R. Ponziani MD, UOC Internal Medicine and Gastroenterology, Policlinico A. Gemelli, Catholic University of Rome, largo A. Gemelli 8 00168, Rome, Italy.
Tel.: +393471227242;
fax: +390630157249;
e-mail: francesca.ponziani@yahoo.it

Conflicts of Interest
The authors have no conflict of interest to disclose.

Received: 13 June 2012
Revision requested: 17 July 2012
Accepted: 1 November 2012
Published online: 11 December 2012

doi:10.1111/tri.12027

Summary

The aim of this study was to evaluate the factors affecting the response to treatment and how it could affect survival in a large series of genotype-1 HCV-transplanted patients. Three-hundred and twenty six genotype-1 HCV patients were enrolled. One hundred and ninety-six patients (60.1%) were nonresponders and 130 (39.9%) showed negative HCV-RNA at the end of treatment. Eighty-four of them (25.8%) achieved sustained virological response, while 46 (14.1%) showed viral relapse. Five-year cumulative survival was significantly worse in nonresponders (76.4%) compared with sustained viral response (93.2) or relapsers (94.9%). Sustained responders and relapsers were therefore considered as a single 'response group' in further analysis. Pretreatment variables significantly associated with virological response at multivariate regression analysis were the absence of ineffective pretransplant antiviral therapy, the recurrence of HCV-hepatitis more than 1 year after transplant, an histological grading ≥ 4 at pretreatment liver biopsy, a pretreatment HCV-RNA level $<1.2 \times 10^6$ IU/ml, and the absence of diabetes. As expected, also on-treatment variables (rapid and early virological response) were significantly associated to the response to antiviral treatment. In conclusion, this study shows that postliver transplant antiviral treatment results in beneficial effect on survival not only in sustained responders but also in relapsers.

Introduction

Hepatitis C virus (HCV)-related cirrhosis is a major indication for liver transplantation (LT) worldwide [1]. An histological pattern of recurrent hepatitis is observed in more than 70% of those recipients, and HCV infection, together with the presence in the recipient of the allele 4 of Cytochrome P2D6, have been shown to be the main risk

factors for liver fibrosis progression in transplanted patients [2]. Development of cirrhosis can be observed in about 30% of patients transplanted for HCV-related cirrhosis at 5 years from LT. Once cirrhosis is established, up to 42% of patients develop liver decompensation within 1 year [3–5].

Anti-viral therapy (AT) is based on standard or pegylated alpha-interferon (PEG-IFN) plus ribavirin (RBV), and it is performed after LT at the time of histological HCV-hepatitis recurrence. Sustained viral response (SVR) rates have been reported to range between 30% and 48% [6–9]. As in non-LT setting, higher SVR rates in genotype 2/3 than in genotype 1/4 patients are reported [9], and vitamin D deficiency has been recently shown to predict an unfavourable response to AT [10].

The impact of AT on patients' survival is still controversial. The likelihood of developing cirrhosis seems to be reduced in patients achieving SVR and 5-year survival has been shown to be higher in SVR than in untreated [6] or in nonresponder (NR) recipients [9,11]. However, a systematic review [12] on 12 prospective randomized studies, including overall 425 patients with mainly genotype-1 (G1) HCV-hepatitis, did not report any significant difference in mortality or re-transplantation rates between patients who underwent AT and those who did not. The limit of this study was that the mean patients' follow-up after the end of AT was about 6 months; moreover, in none of the considered studies data on long-term patient or graft survival were reported.

In this study, we have assessed a large series of patients from 12 Italian transplant centres with post-LT G1 HCV-hepatitis recurrence treated with AT, to establish the rate of response, the predictors of viral response and the impact of viral response on long-term cumulative survival.

Patients and methods

Three hundred and twenty-six G1-HCV LT-recipients, transplanted between January 1999 and December 2008 in 12 Italian transplant centres, who underwent AT for G1 HCV-hepatitis recurrence were included in the analysis.

The main clinical and laboratory patients' characteristics are reported in the first column of Table 1. In all patients, the recurrence of HCV-hepatitis was suspected on the basis of biochemical findings (raised serum ALT levels with or without increased cholestasis parameters) and confirmed by liver biopsy performed no more than 3 months prior to AT beginning. Histology was evaluated by Ishak scoring system [13]. None of the patients showed clinical or histological findings suggestive of cholestatic fibrosing hepatitis. In all patients, the diagnosis of diabetes was made following the internationally accepted criteria available since 1997 [14].

In 142 patients, AT was started within 1 year, in 77 between 1 and 2 years and in 107 more than 2 years after LT. One-third of patients (33.4%) had failed to respond to a previous pretransplant AT ('non-naïve' patients).

A serum HCV-RNA determination was available both prior to and at the end of AT and 6 months after AT completion; in 160 patients it was also available at 4 weeks, and in 276 patients at 12 weeks after the start of AT. Quantitative HCV-RNA using polymerase chain reaction assays (Amplicor, Roche, Switzerland; version commercially available at the time of serum assay), were available in 284/326 patients (87.1%); mean pre-AT viraemia was $5.9 \pm 0.82 \times 10^6$ IU/ml (range $0.0001–40 \times 10^6$ IU/ml). In the remaining patients, only a qualitative positive serum HCV-RNA was available before AT.

Two hundred and forty-five patients (75%) were treated with recombinant PEG-IFN alpha2a (180 μg s.c. once weekly) or 2b (1.0 to 1.5 μg/kg body weight s.c. once weekly) plus RBV, for a scheduled period of 48 weeks. RBV was administered orally twice daily, at dosages ranging between 200 and 1200 mg/day, according to patients' weight, tolerance and creatinine clearance values. Eighty-one patients, mainly treated before 2003, received standard IFN alpha2b (3 million units s.c. thrice weekly). RBV or PEG-IFN dose reduction and granulocyte colony-stimulating factor (GCSF) or erythropoietin administration were performed according to the protocol adopted in each centre. RBV dose was usually reduced at haemoglobin value <10g/dl by 200–400 mg/day, standard or PEG-IFN dose at neutrophil count $<1.0\times 10^9$/l or platelet count $<35\times 10^9$/l; GCSF was usually started at neutrophil count $<0.75\times 10^9$/l and erythropoietin at haemoglobin value <9 g/dl.

The standard post-LT immunosuppressive regimen consisted of steroids (usually withdrawn within 3–6 months from LT) and a calcineurin inhibitor (cyclosporine-A or tacrolimus in 39.8% and 57.7% of the cases respectively). In 41 patients, mycophenolate (mofetil) had been added after reduction of cyclosporine-A or tacrolimus because of the occurrence of side effects.

In absence of detectable HCV-RNA at the end of treatment (EOT) or at treatment withdrawal and 6 months later, patients were considered SVR; those showing a negative HCV-RNA at the EOT or at treatment withdrawal but not maintaining a negative viraemia 6 months later were considered relapsers (REL). The patients not achieving a negative HCV-RNA at the EOT were considered NR.

An undetectable HCV-RNA at week 4 after AT beginning was defined as rapid virological response (RVR), while a negative HCV-RNA (or at least 2-$\log_{10}$ decline in HCV-RNA compared with baseline value) at week 12, was defined as early virological response (EVR).

Anti-viral therapy was discontinued in 145 cases (44.5%). The main causes of therapy withdrawal were myelotoxic effects with peripheral cytopenia unresponsive to IFN and/or RBV reduction and/or administration of erythropoietin

Table 1. Baseline characteristics of the 326 LT-recipients with G1 HCV-hepatitis enrolled in the study (data in mean ± SE; in brackets no. available data).

Parameter	All cases (326)	NR (196)	REL (46)	SVR (84)
Gender	230 M, 96 F (326)	134 M, 62 F (196)	31 M, 15 F (46)	65 M, 19 F (84)
Age at LT (years)	54.3 ± 0.45 (307) (median = 56)	53.7 ± 0.61 (181) (median = 55)	55.6 ± 1.07 (44) (median = 57)	54.8 ± 0.84 (82) (median = 57)
BMI	25.3 ± 0.24 (321) (median = 24.4)	24.7 ± 0.25 (188) (median = 24.1)	26.7 ± 0.75 (44) (median = 25.0)	26.1 ± 0.50 (83) (median = 24.0)
Interval LT–recurrent HCV-hepatitis (months)	7.9 ± 0.60 (252) (median = 4.6)	6.9 ± 0.69 (157) (median = 3.9)	9.9 ± 2.02 (28) (median = 7.3)	9.4 ± 1.29 (67) (median = 5.5)
Pre-LT unsuccessful AT ('non-naïve' patients) (Y/N)	65/130 (195)	44/61 (105)	8/25 (33)	13/44 (57)
Immunosuppression	(324)	(194)	(46)	(84)
Cyclosporin	129	72	20	37
Tacrolimus	187	117	25	45
Other	5	3	0	2
Tolerant	3	2	1	0
HCV-RNA ($\times 10^6$ IU/ml)	5.9 ± 0.82 (284)	6.7 ± 1.23 (170)	5.8 ± 1.32 (39)	4.0 ± 1.19 (75)
ALT (IU/l)	162.9 ± 9.4 (233)	173.0 ± 13.5 (130)	126.7 ± 19.2 (39)	164.6 ± 17.9 (64)
Neutrophil count ($\times 10^9$/l)	2.6 ± 0.09 (177)	2.5 ± 0.11 (105)	2.6 ± 0.27 (32)	2.8 ± 0.22 (40)
Platelet count ($\times 10^9$/l)	149.0 ± 12.9 (233)	155.3 ± 22.9 (134)	143.4 ± 11.0 (27)	137.2 ± 8.6 (52)
Diabetes (Y/N)	109/161 (270)	70/81 (151)	9/26 (35)	25/49 (74)
Histological grading*	6.1 ± 0.12 (267) (median = 6)	5.9 ± 0.26 (154) (median = 6)	6.4 ± 0.45 (38) (median = 6)	6.4 ± 0.37 (75) (median = 6)
Histological grading (≥ 4 vs. <4)*	212/55 (267)	114/40 (154)	35/3 (38)	63/12 (75)
Histological staging*	2.2 ± 0.07 (265) (median = 2)	2.2 ± 0.10 (155) (median = 2)	2.3 ± 0.14 (46) (median = 2)	2.2 ± 0.15 (64) (median = 2)
	Stage 1: 90 (34%)	Stage 1: 55 (35.5%)	Stage 1: 10 (21.7%)	Stage 1: 25 (39.1%)
	Stage 2: 84 (31.7%)	Stage 2: 49 (31.6%)	Stage 2: 19 (41.3%)	Stage 2: 16 (25.0%)
	Stage 3: 52 (19.6%)	Stage 3: 28 (18.1%)	Stage 3: 11 (23.9%)	Stage 3: 13 (20.3%)
	Stage 4: 28 (10.6%)	Stage 4: 16 (10.3%)	Stage 4: 5 (10.9%)	Stage 4: 7 (10.9%)
	Stage 5: 8 (3.0%)	Stage 5: 5 (3.2%)	Stage 5: 1 (2.2%)	Stage 5: 2 (3.1%)
	Stage 6: 3 (1.1%)	Stage 6: 2 (1.3%)	Stage 6: 0	Stage 6: 1 (1.6%)
HCC at LT (Y/N)	74/115 (189)	36/71 (107)	14/16 (30)	24/28 (52)
Type of AT	(326)	(196)	(46)	(84)
Standard IFN/RBV	81	53	10	18
PEG-IFN/RBV	245	143	36	66

NR, nonresponders; REL, relapsers; SVR, sustained virological responders; LT, liver transplant; BMI, body mass index; ALT, alanine aminotransferase; IFN, interferon; RBV, ribavirin.
*According to Ishak *et al.* [13].

or GCSF (22.1% of cases), patients' intolerance to treatment (16.6%), inadequate viral response (12.4%), occurrence of neuropsychiatric symptoms (9.0%), histologically proven acute rejection (8.3%), liver function failure or clinical decompensation (5.5%), autoimmune hepatitis (4.8%), severe bacterial infection (4.8%) and biliary complications (3.4%). The remaining (13.1%) causes of treatment withdrawal were ascribed to less common side effects such as IFN-related retinopathy, acute renal failure, hypertriglyceridaemia, pulmonary embolism, pulmonary hypertension, acute pancreatitis, peripheral neuropathy, ischaemic cardiopathy, cutaneous reaction, hepatic artery thrombosis, hypertriglyceridaemia and patients refusal to complete the planned AT. In spite of treatment discontinuation, 27 of these patients (18.6%) were HCV-RNA negative at the moment of treatment withdrawal, and 14 of them (9.6%) maintained the virological response 6 months later, so achieving a SVR. On the whole, 60.4% of patients received at least 80% of the scheduled AT.

Statistical analysis

To determine the impact of AT on cumulative survival, post-LT and post-AT survival rates in SVR, REL and NR subgroups of patients were calculated by Kaplan–Meier analysis and compared with log-rank test.

To identify the single variables statistically associated to the development of a virological response, the differences

in any of the investigated parameters among SVR, REL and NR subgroups were first evaluated by univariate variance analysis (or chi-squared test for nonparametric variables).

To identify the independent prognostic factors and to establish the statistical 'weight' of each clinical parameter in predicting AT response, all the variables associated (i.e. $P \leq 0.10$) with the response to AT in the previous univariate analysis were then entered in a stepwise multivariate Cox regression model, excluding those variables unable to show an independent prognostic value.

For each independent prognostic factor, the statistical significance (adjusted r^2, partial F-values and probability level) and the standardized weight in the multivariate model were calculated, together with the single coefficients for the multivariate predicting regression analysis, to assess the combination of factors providing the best discrimination between responders (RE) and NR.

Wherever indicated comparison between groups was made using the chi-squared test, a P-value lower than 0.05 was considered significant.

Results

Among the 326 patients, 196 (60.1%) were NR, while 130 (39.9%) showed a negative HCV-RNA at the end of the AT period. Eighty one of these 130 responder patients were SVR, and the remaining 49 were REL. Ten REL patients underwent a further course of AT during the follow-up, three of them achieving a SVR. None of the NR was retreated with a new AT course nor was maintained on long-term IFN therapy. Therefore, on the whole population, 84 patients had SVR, and 46 were REL.

Overall, SVR rate was therefore 25.8%, with no significant differences among the different centres or between high (i.e. >30 LT per year) or low-volume centres. SVR was more frequently achieved in the patients completing more than 80% of the scheduled treatment (27.8% vs. 19.6%) but this difference did not reach statistical significance ($P = 0.15$). However, EVR was significantly more frequent in the patients receiving more than 80% of the scheduled treatment (40.2% vs. 23.3%; $P = 0.017$).

The rate of SVR, REL and NR did not differ significantly when considering the patients included in the study up to December 2003 (24.0%, 16.6% and 59.4% respectively) compared with those enrolled after January 2004 (27.6%, 11.2% and 61.2%) ($P = 0.316$).

Overall, cumulated post-LT survival rates were of 96.6% at 1 year and 84.1% at 5 years. Figure 1 shows the cumulative survival rates of the investigated patients according to the response to AT. Overall, long-term cumulated survival was significantly worse in NR (76.4% at 5 years; relative mortality, RM 1.52) than in SVR (93.2%, RM 0.35) or REL (94.9%, RM 0.33) (log-rank chi-square 27.1; d.f.: 2; $P < 0.0001$). SVR and REL patients did not show any significant difference in long-term cumulative survival.

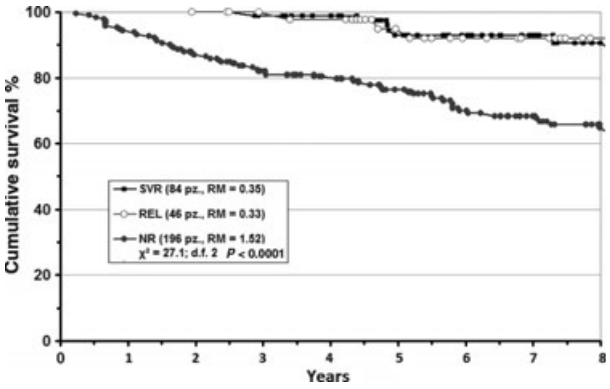

Figure 1 Cumulative post-LT survival rates in SVR (sustained viral response), REL (relapser) and NR (nonresponder) patients.

Similar findings were observed when overall cumulative survival in the three groups was evaluated starting from the time of the onset of antiviral therapy. Five-year post-AT cumulative survival was significantly worse in NR (76.3%; RM 1.44) compared with SVR (89.3%; RM 0.52) or REL (97.1%; RM 0.33) (log-rank chi-square 8.9; d.f.: 2; $P < 0.02$). Median post-AT survival was 88 (range 31–242) months in SVR group, 90 (range 24–182) months in REL group and 71 (range 3–219) months in NR group.

The SVR and REL patients did not show any significant difference either in baseline demographic and virological characteristics (Table 1), no in long-term cumulative survival. On the basis of these results, SVR and REL were considered as a single 'responder' group of patients (RE) in further analysis, to be compared with the NR group only.

Overall median post-LT and post-AT follow-up periods in our cohort of patients were 84 months (range 3–242), and 72 months (range 1–192), respectively. The median post-LT follow-up period of SVR and REL patients was not significantly different (108 months, range 35–259, vs. 109 months, range 30–236). The number of patients died was 86 (26.3%). Of them, 14 were RE, and 72 NR. End-stage liver failure (mostly because of recurrent HCV-related decompensated cirrhosis) was the main cause of death, accounting for as much as 79.1% of the patients died in NR group, and in 21.4% only of the subjects deceased in RE group ($P < 0.0001$). The rates of death-causing end-stage liver failure between SVR subjects (2/84; 2.3%) and REL patients (1/46; 2.1%) were not significantly different ($P = 0.94$).

The pretreatment variables showing a statistically significant association with RE at retrospective univariate statistical analysis (Table 2) were the recurrence of HCV-hepatitis more than 1 year after transplant, a pretreatment HCV-RNA level $<1.2 \times 10^6$ IU/ml, the absence of ineffective pre-LT AT ('naïve' patients), an histological grading

≥ 4 at pretreatment liver biopsy and the absence of diabetes. As expected, also on-treatment variables (RVR and EVR) were highly significant predictors of RE to antiviral treatment.

To identify the independent prognostic factors and to establish the statistical 'weight' of each clinical parameter in predicting AT response, all the variables associated (i.e. $P \leq 0.10$) with the response to AT in the previous univar-

Table 2. Differences between RE and NR patients: multivariate statistical analysis (data in mean ± SE; in brackets no. available data).

				Multivariate analysis		
Parameter	NR group (196 patients)	RE group (130 patients)	Univariate analysis (*)	Partial *F* statistics (*P*-value)	Standardized weight	Adjusted r^2
Donor						
Age (years)	49.7 ± 1.38 (179)	46.1 ± 1.65 (122)	0.068			
Gender (M/F)	93 M 69 F (162)	67 M 44 F(122)	0.691			
Body weight (kg)	71.6 ± 0.99 (123)	72.7 ± 1.12 (89)	0.483			
Body mass index	24.7 ± 0.25 (129)	25.0 ± 0.30 (91)	0.472			
Presence of HBcAb	10.19% (157)	8.43% (83)	0.662			
Total graft ischaemia time (min)	445.4 ± 10.6 (157)	474.9 ± 16.4 (102)	0.115			
Recipient						
Pretreatment variables						
Age at LT (years)	53.7 ± 0.61 (181)	55.1 ± 0.66 (126)	0.111			
Gender (male)	134 M, 62 F (196)	96 M, 34 F (130)	0.271			
Body mass index	24.7 ± 0.25 (194)	26.3 ± 0.41 (127)	0.109			
MELD at LT	17.3 ± 0.51 (153)	17.1 ± 0.50 (96)	0.738			
Pre-LT unsuccessful AT ('non-naïve' patient)	41.9% (105)	23.3% (90)	0.006 (†)	7.14 (†) (0.008)	-0.016	0.113
Interval LT-recurrent HCV-hepatitis (months)	6.9 ± 0.69 (157)	9.5 ± 1.08 (95)	0.037 (†)			
Interval LT-recurrent HCV-hepatitis > 1 year	12.9% (155)	33.7% (95)	0.001 (†)	8.49 (†) (0.004)	0.229	0.076
Genotype (1a/1b)	16/173	7/116	0.322			
Creatinine (mg/dl)	1.14 ± 0.08(154)	1.12 ± 0.03 (105)	0.850			
ALT (IU/l)	173.0 ± 13.5 (130)	150.2 ± 13.1 (103)	0.241			
HCV-RNA ($\times 10^6$IU/ml)	6.7 ± 1.23 (170)	4.6 ± 0.99 (114)	0.215			
HCV-RNA > 1.2×10^6 IU/ml	62.9% (170)	49.1% (114)	0.002(†)	4.82 (†) (0.029)	-0.211	0.013
Histological grading	5.9 ± 0.26 (154)	6.4 ± 0.29 (113)	0.168			
Histological grading ≥ 4	74.0% (154)	86.7% (113)	0.011(†)	8.32 (†) (0.004)	0.120	0.042
Histological staging	2.1 ± 0.10 (155)	2.3 ± 0.11 (110)	0.353			
Presence of HBcAb	29.6% (142)	19.8% (91)	0.096			
Presence of diabetes	46.6% (151)	31.2% (109)	0.011(†)	5.72 (†) (0.0175)	-0.04	0.017
Serum ferritin (mg/ml)	372.3 ± 42.1(84)	291.3 ± 37.3 (60)	0.172			
Neutrophil count ($\times 10^9$/l)	2.5 ± 0.11 (105)	2.7 ± 0.17 (72)	0.209			
Platelet count ($\times 10^9$/l)	155.3 ± 22.9 (134)	139.3 ± 6.7 (79)	0.593			
Immunosuppressive regimen	(194)	(130)				
Cyclosporine	72	57	0.27			
Tacrolimus	117	70				
Other	3	2				
Immunotolerant	2	1				
Type of AT	(196)	(130)	0.22			
Standard IFN/RBV	53	28				
PEG-IFN/RBV	143	102				
On-treatment variables						
RVR	4.6% (87)	20.6% (73)	0.0018 (†)			
EVR	11.9% (159)	76.9% (117)	0.0001 (†)			

NR, nonresponders; REL, relapsers; SVR, sustained virological responders; LT, liver transplant; BMI, body mass index; ALT, alanine aminotransferase; IFN, interferon; RBV, ribavirin; RVR, rapid virological response; EVR, early virological response.

*One-way variance analysis (chi-squared test for categorical variables) probability.

†Statistically significant.

iate analysis were then entered in a stepwise multivariate regression model. The multivariate analysis confirmed that the variables independently associated to the individual response to AT at multivariate analysis (Table 2) were the absence of ineffective pretransplant AT ('naïve' patients), the recurrence of HCV-hepatitis more than 1 year after transplant, a histological grading ≥ 4 at pretreatment liver biopsy, a pretreatment HCV-RNA level $<1.2 \times 10^6$ IU/ml and the absence of diabetes.

The multiple regression analysis performed with all the pretreatment variables independently associated to the individual response to AT showed a highly significant statistical value ($F = 4.98$; d.f. 4 and 121; $P < 0.001$).

Discussion

To our knowledge, this is the largest study assessing the effectiveness of combined AT outcome in patients with G1-HCV, that accounts for more than 85% of the post-LT recurrent HCV-hepatitis in the available series [15].

The achievement of SVR was observed in 25.8% of our patients. Other authors reported SVR rates of 12.5–40% in G1 patients, the highest (40%) in series including a low number of patients [16, 17].

The observed SVR rate in our patients is consistent with that (26%) reported in a single-centre Italian study concerning 53 transplanted subjects with recurrent G1 HCV-hepatitis [18], confirming that only a minority of G1-HCV patients may achieve SVR after LT.

However, despite a low rate, the achievement of SVR was associated with a significantly higher patient's survival compared with that of NR patients (93.2% vs. 76.4%) (Fig. 1). This result confirms what reported in other retrospective studies including a minority of patients with HCV recurrence because of other viral genotypes [9, 11]. The better survival observed in SVR patients might be related to the improvement of liver necro-inflammation and fibrosis progression, with the consequent drastic reduction of cirrhosis development and liver-related mortality [1].

The interesting finding of this study is that a beneficial effect of the AT on survival was fairly evident not only in SVR but also in REL patients, who showed a 5-year survival of 94.9% that did not differ significantly from that of SVR (Fig. 1). A similar beneficial effect of AT on survival in both SVR and in REL has been observed by other authors in smaller series including other HCV genotypes too [9, 19]. This could imply that the achievement of an EOT virological response by itself, independently of its maintenance over time, may improve the natural history of recurrent hepatitis and exert a favourable effect on long-term survival by decreasing the progression of fibrosis, and the development of cirrhosis. The reduced number of deaths because of end-stage liver disease we observed among SVR and REL patients compared with NR ones further supports this conclusion. Furthermore, it is well-known that, in the natural history of post-LT HCV-hepatitis, severe necro-inflammatory activity at liver histology is predictive of cirrhosis development [20]. Accordingly, compared with pre-AT histological findings, the aforementioned study by Jain *et al.* showed a relevant reduction in hepatic activity index not only in SVR but also in REL on liver biopsy specimens obtained after the end of AT [19]. In this study, liver biopsies performed at 3 years from AT completion were available in 15 SVR, 10 REL and 24 NR patients. With respect to pre-AT values, we arbitrarily divided grading and staging scores according to Ishak *et al.* [13] into 'unchanged' (i.e. unmodified score point), 'improved' (i.e. score lower than pre-AT) or 'worsened' (i.e. score higher than pre-AT). The occurrence of grading improvement did not differ significantly between REL (7/10 pts, 70%) and SVR patients (11/15, 73.3%) ($P = 0.337$), while staging improvements were observed in SVR (8/15, 53.3%) but not in REL patients (in whom the score was unchanged in all cases) ($P = 0.004$). Both SVR and REL patients showed significant differences in histology progression with respect to NR patients, who showed lower rates of grading improvement (9/24, 37.5%) compared both to SVR (73.3%, $P = 0.049$) and REL subjects (70.0%, $P = 0.034$). Moreover, the staging score in the NR group was worsened in 15 cases (62.5%), and this rate was significantly higher than that found in SVR (2/15, 13.3%, $P = 0.0006$) or REL (none of the patients, $P = 0.001$) patients. These data, although referring to a small subgroup of our patients only, seem to further support the hypothesis of a treatment-related decreased necro-inflammatory and fibrosing activity not only in SVR but also in REL patients.

Predictors of viral response

Pretreatment parameters

As far as the recipient's virological status is concerned, the absence of a pre-LT AT (performed in 23.3% of RE and in 41.9% of NR) was a significant predictor of response. We could therefore confirm that the probability to have a good response to re-AT of recurrent HCV-hepatitis in recipients who have already failed to respond to an adequate course of PEG-IFN plus RBV before LT should be expected to be low [21].

Furthermore, in agreement with other Authors, we observed that a lower viral load before AT was associated to a higher probability of SVR [11, 16, 22]. Accordingly, it could be reasonable to adopt a pre-AT schedule with drugs able to decrease HCV viral load in LT-recipients with high HCV baseline viraemia. Recently, a 10-day pre-AT with intravenous silibinin has been shown to significantly decrease viral load before a course of PEG-IFN plus RBV

therapy in four previously nonresponders transplanted patients with recurrent HCV-hepatitis [23]. Another promising approach could be a pre-AT period with RBV alone. In a recent pilot study, performed in 13 LT-recipients with recurrent HCV-hepatitis, a 8-week RBV priming period before combination therapy provided a significant decrease in median HCV-RNA level, and a decline of 0.5 $\log_{10}$ HCV-RNA during pre-AT predicted the achievement of RVR [24].

In this study, RE showed a higher grading of necro-inflammatory activity. Both a high degree of necro-inflammation [9], and a baseline activity score higher than 5 [25] have been reported as significant predictors of SVR achievement. On the contrary, liver fibrosis staging was not a significant predictor of response. Consistently with our results, low baseline fibrosis was found to be associated with the achievement of SVR only in two of 10 studies investigating baseline fibrosis stage as a potential predictive factor and in none of these studies this variable resulted significant at the multivariate analysis [15]. However, it must be pointed out that, in the nontransplant setting, cirrhotic patients with HCV-related disease show a low likelihood of responding to AT [26], and that our series included only a few patients with histologically proven cirrhosis (4.1% of the cases).

RE patients showed a delayed occurrence of post-LT recurrent hepatitis compared with NR, suggesting that an earlier viral relapse might be more severe and more difficult to treat than a later one. Our results support the conclusion of Oton *et al.*, showing that the recurrence of HCV-hepatitis later than 2–4 years after LT is a significant predictive factor of SVR [16].

Finally, this is the first study reporting the presence of type-2 diabetes as a significant risk factor for nonresponse in LT-patients undergoing AT. The risk of developing diabetes mellitus has been shown to be increased in patients with chronic HCV infection compared with control individuals or patients with hepatitis B virus [27]; in addition, chronic HCV infection has been associated with insulin resistance [28], and insulin resistance has been identified as a risk factor for fibrosis severity in chronic HCV-hepatitis [29]. In LT setting, approximately half of HCV-positive recipients develop post-transplant diabetes mellitus, which is associated with poorer graft and patient survival outcomes [30]. In nontransplanted patients with HCV-hepatitis, insulin resistance and diabetes mellitus are predictors of inadequate response to antiviral treatment [31]. In a recent paper performed in a large cohort of patients with chronic HCV-hepatitis submitted to antiviral therapy, impaired fasting glucose and/or diabetes mellitus were associated with lower SVR rate [32]. Another study assessed the association of diabetes with the severity of hepatic steatosis/fibrosis, and with the response to HCV AT; in comparison with nondiabetics, diabetic patients were more likely to have liver steatosis and advanced fibrosis, and achieved SVR in a significantly lower proportion of cases (23% vs. 46%) [33]. This study seems to confirm that diabetes mellitus adversely affects response to AT also in LT-recipients with recurrent HCV-hepatitis.

On-treatment parameters

A sustained response to AT was detected in 78.9% of patients achieving RVR, and in only 41.1% of cases without RVR, with a RVR rate of only 4.6% in NR. RVR has been already shown to be a predictive factor of SVR in LT-recipients [16, 34]; its achievement allows to shorten the AT duration to 24 weeks in non-LT G1 patients with HCV-RNA serum level lower than 0.4–0.8 MIU/ml [26]. The intriguing possibility to shorten the AT also in G1 LT-patients with recurrent hepatitis and low pre-AT viraemia who achieve a RVR should be investigated in future studies.

The achievement of EVR represented the most reliable predictor of AT response, because SVR was observed in 82.6% of patients achieving EVR, and in 16.2% of those without EVR, with an EVR rate of 11.9% only in NR subjects. Similar to the non-LT setting, several studies highlighted that an undetectable HCV-RNA or a drop of HCV-RNA more than 2 $\log_{10}$ at week 12 represent the most important tools to predict successful AT outcome in LT-recipients [6–9, 16, 17, 35–38]. This study confirms these data suggesting that in all patients achieving an EVR, all efforts should be made to complete the scheduled therapy and to avoid AT withdrawal because of side effects. However, in some patients without an EVR but showing histological findings of cholestatic hepatitis and biochemical response (e.g. persistently normal ALT serum level) a benefit might be derived from treatment continuation to delay the progression of necroinflammation. Furthermore, maintenance therapy with low dose-dose PEG-IFN in patients who do not achieve SVR but normalize the transaminases during the treatment could provide a histological benefit and limit hepatic deterioration over time [15]. This approach has been shown to be ineffective in the immunocompetent population [39], but its value should be reassessed in the different immunological context of LT.

This study presents some limits. First of all, AT drop-out rules and immunosuppressive regimens were not fully standardized, being adopted in each centre on a patient by patient basis. Second, the histological evaluation of liver biopsies and the HCV-RNA assays were not centralized. Third, we could not provide data about the interleukin 28B genotype of both the recipient and donor liver which has been recently shown to be predictive of SVR achievement, being significantly higher for CC compared with CT/TT genotypes [40, 41]. Finally, this is a retrospective survey with all its inherent limits and some unavoidable selection biases.

In conclusion, we were able to confirm that G1 patients with post-LT recurrent hepatitis submitted to combined treatment with IFN and RBV achieve a SVR only in a minority of cases, and that SVR is associated with a beneficial effect on cumulative survival of treated patients. In non-transplanted G1 patients the introduction of protease inhibitor-based regimens has increased substantially the rate of SVR achievement but no robust data are still available about both safety and efficacy of triple therapy in transplanted HCV patients. In addition, this study demonstrates an AT-related positive impact on survival also in REL patients. A number of pre-AT factors related to patients' virological status (no AT before LT, low viral load, late hepatitis recurrence), liver histology (high necro-inflammatory grading) and metabolic competence (absence of diabetes), were associated to the achievement of virological response. Finally, we could confirm that AT may be reasonably withdrawn in G1 LT-patients who do not achieve EVR, which is a very reliable predictor of virological response.

Authorship

FRP: collected data and managed data collection, wrote the article. AM and RZ: performed statistical analysis and wrote the article. MPAG: wrote and revised the article. RV, RMI, MFD, MR, PT, LP, MC, PB, LM, MM and DDP: participated to data collection and revised the article. SF: managed data collection and revised the article.

Funding

The authors have declared no funding.

Acknowledgements

Thanks to the colleagues collaborating with the AISF RECOLT-C group: Belli L, Gerunda GE, Marino M, Montalti R, Di Benedetto F, De Ruvo N, Rigamonti C, Colombo M, Rossi G, Di Leo A, Lupo L, Memeo V, Bringiotti R, Zappimbulso M, Bitetto D, Vero V, Colpani M, Fornasiere E, Pinna AD, Morelli MC, Bertuzzo V, De Martin E, Senzolo M, Ettorre GM, Visco-Comandini U, Antonucci G, Angelico M, Tisone G, Giannelli V, Giusto M.

References

1. Guillouche P, Feray C. Systematic review: anti-viral therapy of recurrent hepatitis C after liver transplantation. *Aliment Pharmacol Ther* 2011; **33**: 163.
2. Zimmermann T, Hoppe-Lotichius M, Körner A, *et al.* The recipient CYP2D6 allele 4-associated poor metabolizer status correlates with an early fibrosis development after liver transplantation. *Transpl Int* 2011; **24**: 1059.
3. Feray C, Shouval D, Samuel D. Will transplantation of an hepatitis C-infected graft improve the outcome of liver transplantation in HCV patients? *Gastroenterology* 1999; **117**: 263.
4. Berenguer M, Ferrell L, Watson J, *et al.* HCV-related fibrosis progression following liver transplantation: increase in recent years. *J Hepatol* 2000; **32**: 673.
5. Neumann UP, Berg T, Bahra M, *et al.* Fibrosis progression after liver transplantation in patients with recurrent hepatitis C. *J Hepatol* 2004; **41**: 830.
6. Berenguer M, Palau A, Aguilera V, Rayon JM, Juan FS, Prieto M. Clinical benefits of antiviral therapy in patients with recurrent hepatitis C following liver transplantation. *Am J Transplant* 2008; **8**: 679.
7. Berenguer M, Palau A, Fernandez A, *et al.* Efficacy, predictors of response, and potential risks associated with antiviral therapy in liver transplant recipients with recurrent hepatitis C. *Liver Transpl* 2006; **12**: 1067.
8. Dumortier J, Scoazec JY, Chevallier P, Boillot O. Treatment of recurrent hepatitis C after liver transplantation: a pilot study of peginterferon alfa-2b and ribavirin combination. *J Hepatol* 2004; **40**: 669.
9. Selzner N, Renner EL, Selzner M, *et al.* Antiviral treatment of recurrent hepatitis C after liver transplantation: predictors of response and long-term outcome. *Transplantation* 2009; **88**: 1214.
10. Bitetto D, Fabris C, Fornasiere E, *et al.* Vitamin D supplementation improves response to antiviral treatment for recurrent hepatitis C. *Transpl Int* 2011 Jan; **1**: 43.
11. Picciotto FP, Tritto G, Lanza AG, *et al.* Sustained virological response to antiviral therapy reduces mortality in HCV reinfection after liver transplantation. *J Hepatol* 2007; **46**: 459.
12. Gurusamy KS, Tsochatzis E, Xirouchakis E, Burroughs AK, Davidson BR. Antiviral therapy for recurrent liver graft infection with hepatitis C virus. *Cochrane Database Syst Rev* 2010; **20**: CD006803.
13. Ishak K, Baptista A, Bianchi L, *et al.* Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696.
14. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 1997; **20**: 1183–1197.
15. Berenguer M. Systematic review of the treatment of established recurrent hepatitis C with pegylated interferon in combination with ribavirin. *J Hepatol* 2008; **49**: 274.
16. Oton E, Barcena R, Moreno-Planas JM, *et al.* Hepatitis C recurrence after liver transplantation: Viral and histologic response to full-dose PEG-interferon and ribavirin. *Am J Transplant* 2006; **6**: 2348.
17. Angelico M, Petrolati A, Lionetti R, *et al.* A randomized study on Peg-interferon alfa-2a with or without ribavirin in liver transplant recipients with recurrent hepatitis C. *J Hepatol* 2007; **46**: 1009.
18. Lodato F, Berardi S, Gramenzi A, *et al.* Clinical trial: peg-interferon alfa-2b and ribavirin for the treatment of

genotype-1 hepatitis C recurrence after liver transplantation. *Aliment Pharmacol Ther* 2008; **28**: 450.
19. Jain A, Sharma R, Ryan C, *et al.* Response to antiviral therapy in liver transplant recipients with recurrent hepatitis C viral infection: a single center experience. *Clin Transplant* 2010; **24**: 104.
20. Guido M, Fagiuoli S, Tessari G, *et al.* Histology predicts cirrhotic evolution of post transplant hepatitis C. *Gut* 2002; **50**: 697.
21. Shiffman ML. Treating chronic hepatitis C virus after liver transplantation: balancing the risks against the chance for success. *Liver Transpl* 2007; **13**: 1088.
22. Fernandez I, Meneu JC, Colina F, *et al.* Clinical and histological efficacy of pegylated interferon and ribavirin therapy of recurrent hepatitis C after liver transplantation. *Liver Transpl* 2006; **12**: 1805.
23. Neumann UP, Biermer M, Eurich D, Neuhaus P, Berg T. Successful prevention of hepatitis C virus (HCV) liver graft reinfection by silibinin mono-therapy. *J Hepatol* 2010; **52**: 951.
24. Merli M, Giannelli V, Gentili F, *et al.* Ribavirin priming improves the virological response to antiviral treatment in transplanted patients with recurrent hepatitis C: a pilot study. *Antivir Ther* 2011; **16**: 879.
25. Toniutto P, Fabris C, Fumo E, *et al.* Pegylated versus standard interferon-alpha in antiviral regimens for post-transplant recurrent hepatitis C: Comparison of tolerability and efficacy. *J Gastroenterol Hepatol* 2005; **20**: 577.
26. EASL. Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245.
27. White DL, Ratziu V, El-Serag HB. Hepatitis C infection and risk of diabetes: a systematic review and meta-analysis. *J Hepatol* 2008; **49**: 831.
28. Hui JM, Sud A, Farrell GC, *et al.* Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression. *Gastroenterology* 2003; **125**: 1695.
29. Vanni E, Abate ML, Gentilcore E, *et al.* Sites and mechanisms ofinsulin resistance in nonobese, nondiabetic patients with chronic hepatitis C. *Hepatology* 2009; **50**: 697.
30. Gane EJ. Diabetes mellitus following liver transplantation in patients with hepatitis Cvirus: risks and consequences. *Am J Transplant* 2012; **12**: 531.
31. Romero-Gomez M, Fernandez-Rodriguez CM, Andrade RJ, *et al.* Effect of sustained virological response to treatment on the incidence of abnormal glucose values in chronic hepatitis C. *J Hepatol* 2008; **48**: 721.
32. Moucari R, Asselah T, Cazals-Hatem D, *et al.* Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. *Gastroenterology* 2008; **134**: 416.
33. Elgouhari HM, Zein CO, Hanouneh I, Feldstein AE, Zein NN. Diabetes mellitus is associated with impaired response to antiviral therapy in chronic hepatitis C infection. *Dig Dis Sci* 2009; **54**: 2699.
34. Biselli M, Andreone P, Gramenzi A, *et al.* Pegylated interferon plus ribavirin for recurrent Hepatitis C infection after liver transplantation in naive and non-responder patients on a stable immunosuppressive regimen. *Dig Liver Dis* 2006; **38**: 27.
35. Castells L, Vargas V, Allende H, *et al.* Combined treatment with pegylated interferon (alpha-2b) and ribavirin in the acute phase of hepatitis C virus recurrence after liver transplantation. *J Hepatol* 2005; **43**: 53.
36. Carrion JA, Navasa M, Garcia-Retortillo M, *et al.* Efficacy of antiviral therapy on hepatitis C recurrence after liver transplantation: a randomized controlled study. *Gastroenterology* 2007; **132**: 1746.
37. Sharma P, Marrero JA, Fontana RJ, *et al.* Sustained virologic response to therapy of recurrent hepatitis C after liver transplantation is related to early virologic response and dose adherence. *Liver Transpl* 2007; **13**: 1100.
38. Zimmerman MA, Trotter JF, Wachs M, *et al.* Predictors of long-term outcome following liver transplantation for hepatocellular carcinoma: a single-center experience. *Transpl Int* 2007; **20**: 747.
39. Di Bisceglie AM, Shiffman ML, Everson GT, *et al.* Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. *N Engl J Med* 2008; **359**: 2429.
40. Fukuhara T, Taketomi A, Motomura T, *et al.* Variants in IL28B in liver recipients and donors correlate with response to peg-interferon and ribavirin therapy for recurrent hepatitis C. *Gastroenterology* 2010; **1395**: 1577.
41. Charlton MR, Thompson A, Veldt BJ, *et al.* IL28B polymorphisms are associated with histological recurrence and treatment responsefollowing liver transplantation in patients with HCV infection. *Hepatology* 2010; **53**: 317.