

## Long-term leukocyte natural $\alpha$ -interferon and ribavirin treatment in hepatitis C virus recurrence after liver transplantation

Mariarosa Tamè, Federica Buonfiglioli, Massimo Del Gaudio, Andrea Lisotti, Paolo Cecinato, Antonio Colecchia, Francesco Azzaroli, Antonietta D'Errico, Rosario Arena, Claudio Calvanese, Chiara Quarneti, Giorgio Ballardini, Antonio Daniele Pinna, Giuseppe Mazzella

Mariarosa Tamè, Federica Buonfiglioli, Andrea Lisotti, Paolo Cecinato, Antonio Colecchia, Francesco Azzaroli, Rosario Arena, Claudio Calvanese, Chiara Quarneti, Giorgio Ballardini, Giuseppe Mazzella, Department of Digestive Diseases and Internal Medicine, S. Orsola-Malpighi Hospital, University of Bologna, 40138 Bologna, Italy

Massimo Del Gaudio, Antonio Daniele Pinna, Department of Liver and Multiorgan Transplant Unit, S. Orsola-Malpighi Hospital, University of Bologna, 40138 Bologna, Italy

Antonietta D'Errico, Department of Hematology, Oncology and Laboratory Medicine, S. Orsola-Malpighi Hospital, University of Bologna, 40138 Bologna, Italy

**Author contributions:** Tamè M and Buonfiglioli F contributed equally to this work; Mazzella G designed the research; Tamè M and Buonfiglioli F performed the research; Del Gaudio M and Colecchia A performed all liver biopsies; Mazzella G analyzed the data; Tamè M, Buonfiglioli F, Lisotti A and Azzaroli F wrote the paper; Cecinato P, Arena R and Calvanese C performed clinical follow-up of enrolled patients; D'Errico A evaluated liver histology; Quarneti C and Ballardini G contributed immunohistochemistry analysis; Pinna AD coordinated post-liver transplantation management.

**Correspondence to:** Federica Buonfiglioli, MD, Department of Digestive Diseases and Internal Medicine, S. Orsola-Malpighi Hospital, Via Massarenti, 9, 40138 Bologna, Italy. fedebuo82@yahoo.it

Telephone: +39-51-6363888 Fax: +39-51-6364112

Received: February 3, 2013 Revised: June 11, 2013

Accepted: July 4, 2013

Published online: August 28, 2013

(HCV) recurrence received 3 MU three times a week of In- $\alpha$ -IFN plus RBV for 1 mo; then, patients with good tolerability ( $n = 30$ ) were switched to daily IFN administration, while the remaining were treated with the same schedule. Patients have been treated for 12 mo after viral clearance while non-responders (NR) entered in the long-term treatment group. Liver biopsies were planned at baseline, 1 year after sustained virological response (SVR) and at 36 mo after start of therapy in NR. MedCalc software package was used for statistical analysis.

**RESULTS:** About 16.7% of genotype 1-4 and 70% of genotype 2-3 patients achieved SVR. Nine patients withdrew therapy because of non-tolerance or non-compliance. A significant improvement in serum biochemistry and histological activity was observed in all SVR patients and long-term treated; 100% of patients with SVR achieved a histological response (fibrosis stabilization or improvement) with a significant reduction in mean staging value (from 2.1 to 1.0;  $P = 0.0031$ ); histological response was observed in 84% of long-term treated patients compared to 57% of drop-out. Six patients died during the entire study period (follow-up  $40.6 \pm 7.7$  mo); of them, 5 presented with severe HCV recurrence on enrollment. Diabetes (OR = 0.38, 95%CI: 0.08-0.59,  $P = 0.01$ ), leukopenia (OR = 0.54, 95%CI: 0.03-0.57,  $P = 0.03$ ) and severe HCV recurrence (OR = 0.51, 95%CI: 0.25-0.69,  $P = 0.0003$ ) were variables associated to survival. Long-term treatment was well tolerated; no patients developed rejection or autoimmune disease.

**CONCLUSION:** Long-term treatment improves histology in SVR patients and slows disease progression also in NR, leading to a reduction in liver decompensation, graft failure and liver-related death.

### Abstract

**AIM:** To evaluate the effect of long-term treatment with leukocyte natural  $\alpha$ -interferon (In- $\alpha$ -IFN) plus ribavirin (RBV).

**METHODS:** Forty-six patients with hepatitis C virus

© 2013 Baishideng. All rights reserved.

**Key words:** Hepatitis C virus; Hepatitis C recurrence; Interferon; Ribavirin; Liver transplantation

**Core tip:** Recurrent hepatitis C virus hepatitis is associated with a significant increase in morbidity and mortality of transplanted patients; biochemical and necro-inflammatory improves in transplanted patients who achieved a virological response after a course of antiviral treatment. Although the relative small sample size of our study, we demonstrated the efficacy of long-term antiviral treatment on disease progression despite the virological response, without significant side effects.

Tamè M, Buonfiglioli F, Del Gaudio M, Lisotti A, Cecinato P, Colecchia A, Azzaroli F, D'Errico A, Arena R, Calvanese C, Quarneri C, Ballardini G, Pinna AD, Mazzella G. Long-term leukocyte natural  $\alpha$ -interferon and ribavirin treatment in hepatitis C virus recurrence after liver transplantation. *World J Gastroenterol* 2013; 19(32): 5278-5285 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i32/5278.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i32.5278>

## INTRODUCTION

Hepatitis C virus (HCV) -related end-stage liver disease is the main indication for liver transplantation (LT) in Western countries<sup>[1]</sup>. However, graft re-infection is almost universal, leading to accelerated, severe liver disease with a 30% rate of graft cirrhosis after 5 years<sup>[2,3]</sup>. Antiviral treatment is indicated for all patients with evidence of recurrent HCV hepatitis<sup>[4]</sup>; patients with signs of severe HCV recurrence, such as fibrosing cholestatic hepatitis (FCH), must be treated because of the aggressive disease course. The combination of interferon (IFN)- $\alpha$  (both standard and pegylated) plus ribavirin (RBV) is the treatment of choice; however, in the transplant setting, antiviral therapy is less effective. Indeed, IFN plus RBV combination therapy leads to a sustained virological response (SVR) rate of 17%-30%<sup>[5,6]</sup>. PEG-IFN plus RBV treatment has an SVR rate of approximately 30%<sup>[7-9]</sup>, while in immunocompetent patients, the SVR rate ranges from 40%-82% according to the viral genotype<sup>[10]</sup>. The decreased efficacy of antiviral treatment in post-transplant patients may be explained by the low tolerability and the high rate of dose reduction and therapy discontinuation due to adverse events<sup>[5]</sup>. As previously reported, PEG-IFN-based treatment appears to be associated with more hematological and autoimmune adverse events than natural IFN-based therapy<sup>[11-13]</sup>.

Previous studies<sup>[14,15]</sup> have reported that daily IFN administration leads to good virological and histological outcomes with an acceptable tolerability profile; we hypothesized that daily IFN administration could induce an higher, stable serum IFN concentration, similar to PEG-IFN therapy.

To the best of our knowledge, patients with a SVR

to antiviral treatment have improved biochemical and necro-inflammatory activity, while the effect of antiviral treatment on disease progression in non-responders is still controversial<sup>[16]</sup>. Kornberg *et al*<sup>[17]</sup> first described the effect of long-term IFN and ribavirin treatment in transplant patients; the authors reported that antiviral maintenance treatment could prevent disease progression, leading to improved long-term survival. Walter *et al*<sup>[18]</sup> in their retrospective analysis, confirmed the previous results and reported that even in non-responders, long-term antiviral treatment significantly slowed the progression of fibrosis.

Our study aimed to evaluate the virological and histological effects of long-term leukocyte natural  $\alpha$  IFN (In- $\alpha$ -IFN) plus RBV treatment in patients with recurrent HCV hepatitis.

## MATERIALS AND METHODS

### Patients

From January 2003 to January 2008, 46 patients with recurrent HCV after liver transplantation were prospectively enrolled in our study. The diagnosis of recurrent hepatitis C was made using a combination of biochemical [increase in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of at least 2x the ULN], virological (positive serum HCV-RNA) and histological findings. Patients with evidence of decompensated liver disease, histological evidence of rejection or drug-related injury, HBsAg positivity, HIV positivity, moderate to severe anemia (Hb < 10 g/dL), leukopenia (WBC < 1500/ $\mu$ L), thrombocytopenia (platelet count < 50000), impaired renal function (creatinine clearance < 50 mL/min), significant history of cardiovascular and psychiatric diseases or ongoing alcohol abuse were excluded.

### Treatments

After the diagnosis of recurrent hepatitis C, all of the patients received a standard dose of leukocyte natural  $\alpha$ -IFN (Alfaferone, Alfawasserman, Bologna, Italy), 3 MU three times a week (*tiw*) and ribavirin. After one month of treatment, patients with good tolerance received an increased In- $\alpha$ -IFN dose of 3 MU daily (Group A), while patients with poor tolerance to the antiviral treatment were maintained on *tiw* dosing (Group B). Tolerance to antiviral treatment was evaluated based on hematological side effects and patient compliance.

Patients who achieved undetectable HCV-RNA levels continued treatment for 12 mo after viral clearance. Non-responders and relapsers entered the long-term treatment group and were treated with In- $\alpha$ -IFN plus RBV. The S. Orsola-Malpighi internal review board performed a case-by case evaluation for the use of off-label, daily IFN treatment and long-term antiviral therapy. The patients gave informed consent.

Standard immunosuppressive treatment was prescribed to all of the patients at the S. Orsola-Malpighi Hospital; 7 patients received a cyclosporine-based regi-

**Table 1** Patients' baseline characteristics (mean  $\pm$  SE)

Characteristic	Patients ( <i>n</i> = 46)
Sex (M/F)	30/16
Age (yr)	57.9 $\pm$ 1.28
Time from OLT (mo)	26.3 $\pm$ 5.1
ALT (IU/L)	152.3 $\pm$ 17.8
AST (IU/L)	105.9 $\pm$ 11.5
Gamma-GT (IU/L)	188.7 $\pm$ 39.3
Alkaline phosphatases (IU/L)	364.7 $\pm$ 39.9
Bilirubin (mg/L)	1.7 $\pm$ 0.37
Viral load (log <sub>10</sub> )	6.25 $\pm$ 0.09
Genotypes 1/4 vs 2/3	36/10
F1/F2/F3/F4	13/16/9/8
Fibrosing cholestatic hepatitis	3
Cyclosporine A vs tacrolimus	7/39

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; Gamma-GT: Gamma-glutamyl transpeptidase; M/F: Male/female.

men (CyA), while 39 received a tacrolimus-based one (FK).

Patients who presented with anemia or neutropenia received the scheduled IFN and RBV doses; then, erythropoietin was prescribed when the hemoglobin level fell below 10 g/dL, while granulocyte-colony stimulating factor was administered when the neutrophil count was < 700 mmc. When the anemia or neutropenia did not improve with growth factors, the IFN or RBV dose was reduced.

### Biochemistry and virological assessment

Quantitative and qualitative HCV-RNA (Versant HCV-RNA 3.0 bDNA, and Versant TMA; Bayer Diagnostics) were measured before starting treatment, after 1 mo and every 3 mo for the first year; then, serum HCV-RNA was checked every 6 mo. Routine blood tests (blood cell counts and liver and renal function tests) were performed at baseline and weekly for the first 4 wk and then monthly.

### Liver biopsy

A liver biopsy was performed for all of the patients before enrollment. For patients who achieved a virological response, a liver biopsy was repeated 1 year after the end of treatment; the non-responders and relapsers had a repeat liver biopsy after 30 mo of treatment. The histological staging and grading of chronic, recurrent HCV were evaluated according to the Knodell score<sup>[19]</sup>. The diagnosis and grading of liver allograft rejection were made according to the Banff international consensus<sup>[20]</sup>.

### HCV immunohistochemistry

Five-micron-thick sections of liver tissue were obtained and stored at -80 °C. HCV immunohistochemistry (IHC) was performed as previously described<sup>[21-23]</sup>. Reaction positivity was graded according to the percentage of positive cells divided by the total number of hepatocytes (at least 200 cells/high magnification field)<sup>[21-23]</sup>.

### Definition

A rapid virological response was defined as HCV-RNA

decrease of at least 2 log UI/mL or an undetectable level after 1 mo of treatment. A SVR was defined as a persistently undetectable serum HCV-RNA 6 mo after the end of treatment. The presence of fibrosing cholestatic hepatitis (FCH) or F4 fibrosis on enrollment was considered to be severe, recurrent HCV. A histological response was defined as an improvement or stabilization of liver fibrosis.

### Statistical analysis

The data are expressed as the mean  $\pm$  SE. Group comparisons were calculated using the  $\chi^2$  test, the Mann-Whitney test, the Wilcoxon test, *t* tests (both independent sample *t*-test and paired *t*-test), and an ANOVA when appropriate. Clinical events (SVR, and death) were analyzed using Kaplan Meier curves. Logistic regression was used to detect variables that were independently related to the clinical events. Statistical analysis was performed using the MedCalc package v.11.5 for Windows.

## RESULTS

### Patient characteristics

Forty-six patients (30 males, 57.9  $\pm$  1.28 years old) with post-transplant HCV recurrence were prospectively enrolled; the patients' baseline characteristics are described in Table 1. Eleven patients presented with severe liver disease; 3 patients had FHC, and 8 patients had F4 fibrosis. Thirty-five patients presented with chronic HCV hepatitis with F1-3 fibrosis. Thirty-six patients had HCV genotype 1 or 4, while 10 patients had HCV genotype 2 or 3.

### Treatment

The delay between LT and the initiation of treatment was 26.3  $\pm$  5.1 mo. All of the patients (*n* = 46) received *tim* IFN plus RBV treatment for the first month; then, 30 patients received 3 MU IFN daily plus RBV (Group A), while 16 continued *tim* IFN + RBV treatment (Group B). The mean ribavirin dose during the treatment period was 8.4  $\pm$  0.7 mg/kg per day; there was no difference in the RBV dose between Groups A and B.

### Virological response

Among the entire population, seventeen patients (37%) achieved undetectable HCV-RNA levels during therapy and continued IFN+RBV treatment for 12 mo after viral clearance (mean 20.7  $\pm$  2.5 mo); 4 of them relapsed after discontinuing treatment and were included in the long-term treatment group. Thirteen patients achieved an SVR: 8 of 30 patients (26.7%) in Group A and 5 of 16 (31.2%) in Group B. No difference between the groups was observed (*P* > 0.05).

The SVR rate was significantly higher for those with HCV genotype 2 or 3 than genotype 1 or 4 (70.0% vs 16.7%, respectively *P* = 0.0007); the overall SVR rate was 28.3%. Nine patients were rapid virological responders; among them, 7 had HCV genotype 2 or 3, and 2 had genotype 1 or 4. The variables from the univariate

**Table 2** Variables associated with sustained virological response according to univariate analysis

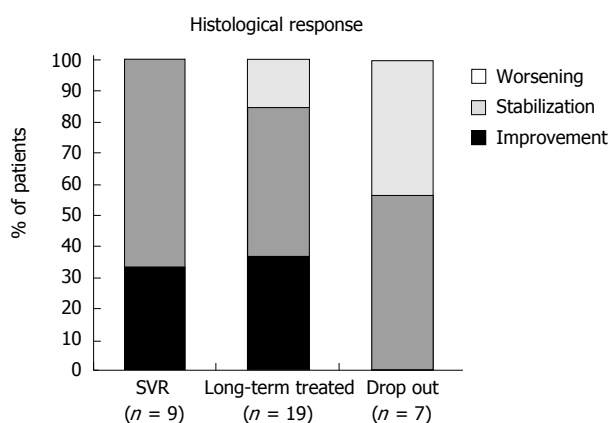
Variable	r	95%CI	P-value
BMI > 25 kg/m <sup>2</sup>	0.30	0.01455-0.5458	0.0400
Genotypes 2-3 vs 1-4	0.37	0.09103-0.5974	0.0100
RVR	0.54	0.2992-0.7194	0.0001
HCV-RNA clearance during treatment	0.82	0.6948-0.8967	0.0001
CyA vs FK immunosuppression	0.41	0.09597-0.6519	0.0120

RVR: Rapid virological response; HCV: Hepatitis C virus; CyA: Cyclosporine; FK: Tacrolimus; BMI: Body mass index.

**Table 3** Histological response after treatment (mean ± SE)

	Grading			Staging		
	Before	After	P-value	Before	After	P-value
Sustained virological response (n = 9)	7.2 ± 0.8	2.6 ± 0.6	0.0039	2.1 ± 0.3	1.0 ± 0.1	0.0031
Long-term treated (n = 19)	7.9 ± 0.7	4.7 ± 0.6	0.0001	2.7 ± 0.3	2.5 ± 0.3	0.0001
Drop out (n = 7)	7.4 ± 1.1	6.0 ± 0.8	NS	2.6 ± 0.6	3.0 ± 0.6	NS

NS: Not significant.



**Figure 1** Histological response. Histological response of patients with sustained virological response, long-term treated and drop-out. SVR: Sustained virological response.

analysis associated with an SVR are shown in Table 2; in the multivariate analysis, a rapid virological response (OR = 99.6, 95%CI: 3.1-3190.0, *P* = 0.0093), a cyclosporine-based immunosuppressive regimen (OR = 685.4, 95%CI: 1.5-314392.9, *P* = 0.036) and the presence of severe, recurrent HCV (OR = 0.91 95%CI: 0.82-0.99, *P* = 0.04) were independently associated with a SVR. Eight patients withdrew therapy after 15.2 ± 2.0 mo, one because of moderate-severe anemia and seven because of non-compliance to therapy. Finally, 25 HCV-RNA-positive patients (21 non-responders and 4 relapsers) entered the long-term treatment group and were treated for a mean of 32.4 ± 2.8 mo.

**Biochemical response**

A significant improvement in ALT was observed in the

13 patients who achieved an SVR (186.1 ± 40.4 IU/L before enrollment vs 21.4 ± 2.2 IU/L after treatment, *P* = 0.0028) and in the 25 long-term treatment patients (154.0 ± 26.6-37.2 ± 4.7 U/L, *P* = 0.0003). Also, those patients who discontinued therapy (*n* = 8) showed a biochemical improvement (ALT 169.5 ± 42.7-58.1 ± 3.6 U/L, *P* = 0.0389). At the end of follow-up, the patients who achieved an SVR had a significantly lower ALT than the other patients (*P* < 0.05). We also observed that the ALT levels were lower in long-term treatment patients compared to the patients who had to stop treatment (*P* < 0.05).

**Histological response**

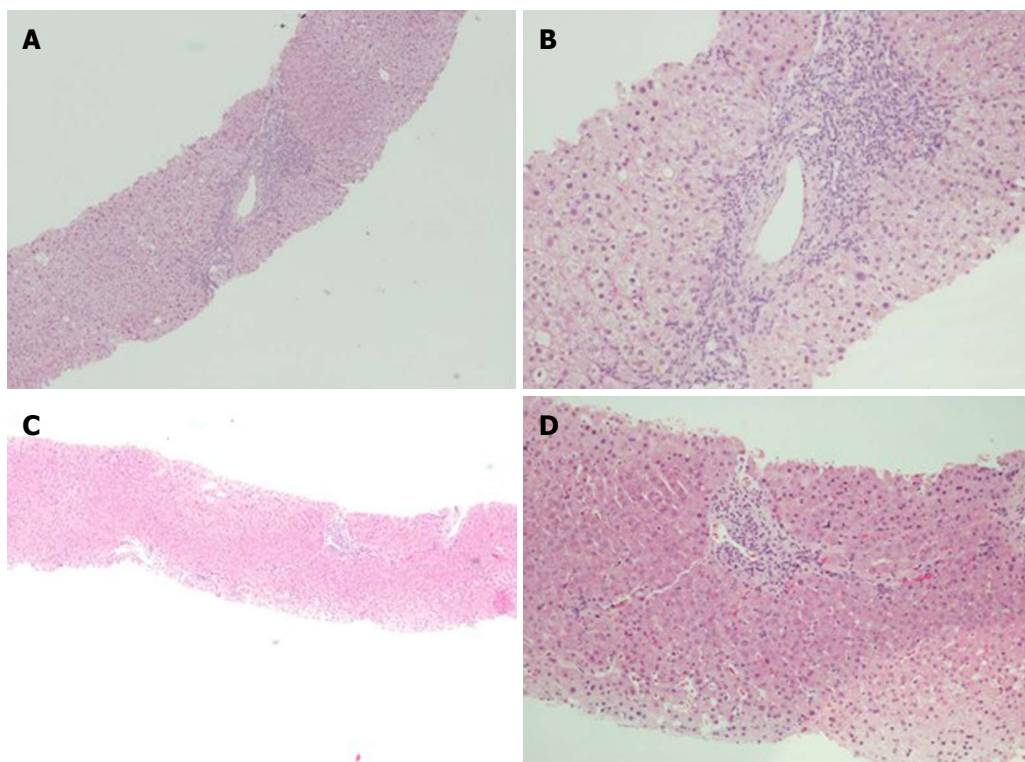
Among the entire population, 35 patients (9 sustained virological responders, 19 long-term treatment patients and 7 who stopped treatment) underwent a second liver biopsy after 30.3 ± 2.7 mo; seven patients refused the paired biopsy, while four died before the scheduled follow-up. The mean grade and stage are shown in Table 3. The post-treatment grade was significantly lower for the patients who achieved a SVR and received long-term treatment (*P* = 0.0039 and 0.0001, respectively), while the grade was unchanged for the patients who discontinued therapy.

Liver fibrosis improved (at least 1 stage) in ten of the 35 (28.6%) patients, remained stable in 19 patients (54.3%) and worsened in six patients (17.1%). The histological response in the three groups (responders, long-term treatment and discontinued treatment) is shown in Figures 1 and 2. Liver fibrosis remained stable or improved (histological response) in all of the patients who achieved a SVR (9 of 9, 100%); in this group, the mean post-treatment fibrosis appeared to be significantly lower (*P* = 0.0031). Interestingly, in the non-sustained virological responders, the histological response was higher in long-term treatment patients (16 of 19) than in the patients who stopped treatment (4 of 7) (84% vs 57%, *P* > 0.05). In the long-term treatment patients, the mean post-treatment fibrosis values were unchanged (2.7 ± 0.3 vs 2.5 ± 0.3).

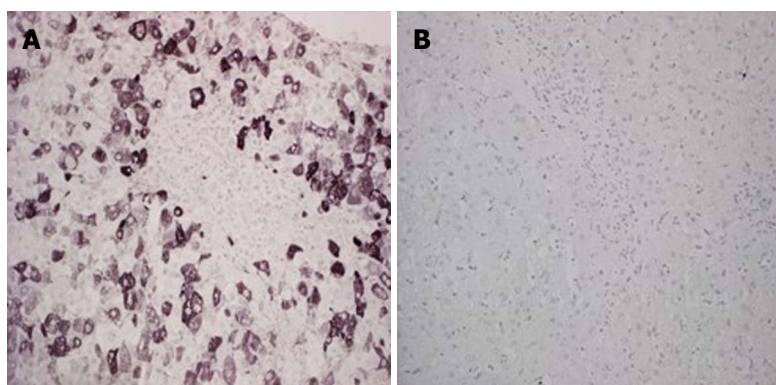
**Liver immunohistochemistry**

HCV IHC (Figure 3) was performed for all of the liver biopsy (46/46) and paired liver biopsy samples (35/35). The median number of immunoreactive hepatocytes before treatment was 50% (95%CI: 38.9-60.0), and there was no significant difference among the responders, long-term treatment patients and patients who stopped treatment (median 60.0%, from 35.3% to 70.0%; median 45.0%, from 17.6% to 68.3%; median 40.0%, from 4.4% to 80.0%, respectively; *P* > 0.05).

After treatment, all of the patients who achieved a SVR (9 of 9) had no (0%) immunoreactive hepatocytes in the liver samples (*P* = 0.0002). Interestingly, the non-responders who received long-term treatment had a significant reduction (*P* = 0.001) in immunoreactive hepatocytes (before treatment: median 45%, from 17.6% to 68.3%; after treatment: median 0.0%, from 0.0% to



**Figure 2 Liver histology.** Liver histology before and after treatment in sustained virological response and Long-term treated patients: liver histology from a patient before treatment (A, B) and liver histology of the same patient (non responder) after long-term treatment (C, D). A, C: Hematoxylin and eosin (HE),  $\times 10$ ; B, D: HE,  $\times 20$ .



**Figure 3 Hepatitis C virus immunohistochemistry.** Lobular areas from serial biopsies of the same patient, showing the cytoplasmic positivity of hepatocyte for hepatitis C virus antigens before (A) and after treatment (B). Immunohistochemistry,  $\times 20$ .

14.1%). No significant difference was observed before and after treatment in the patients who stopped treatment (before: median 40.0%, from 4.4% to 80.0%; after: median 20.0%, from 10.0% to 33.1%;  $P > 0.05$ ).

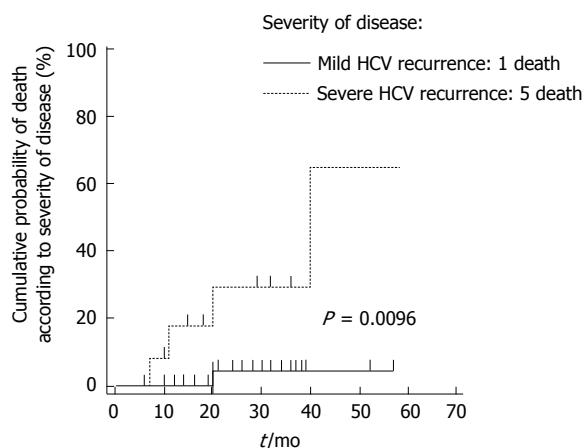
### Tolerability

Treatment was generally well tolerated; 29 (63%) patients, during combination therapy, required growth factors with 28 (61%) patients receiving erythropoietin for anemia and thirteen (28%) receiving G-CSF for neutropenia. Despite the use of grow factors, one patient withdrew from treatment due to moderate-severe anemia (no need for blood transfusion or hospitalization). No patient developed autoimmune disease or graft rejection.

### Survival

Six patients died during the study period (follow-up  $40.6 \pm 7.7$  mo). Two patients with a severe HCV recurrence (FCH), one who was a non-responder and another who stopped therapy, died because of a severe infection (encephalitis and cholangitis). Two patients (basal fibrosis F4), who were non-responders (1 long-term treatment and 1 who stopped treatment), died from liver decompensation. One patient with FCH on enrollment received long-term treatment for 40 mo and died 44 mo after enrollment due to liver decompensation. One patient with a mild HCV recurrence died due to a myocardial infarction.

The presence of diabetes (OR = 0.38, 95%CI: 0.08-0.59;



**Figure 4** Survival analysis (Kaplan-Meier curve) according to presence of severe hepatitis C virus recurrence. HCV: Hepatitis C virus.

$P = 0.01$ ), leukopenia (OR = 0.54, 95%CI: 0.03-0.57;  $P = 0.03$ ) or severe HCV recurrence (OR = 0.51, 95%CI: 0.25-0.69;  $P = 0.0003$ ) were associated with survival; in a multivariate analysis, the presence of a severe HCV recurrence (OR = 29.6, 95%CI: 2.4-371.2;  $P = 0.0086$ ) was the only variable to be independently associated with death. Kaplan-Meier analysis (curve shown in Figure 4) demonstrated an increased risk of death ( $P = 0.0096$ ) for patients with a severe, recurrent HCV compared to patients with a mild recurrence. No difference between the survival of patients undergoing long-term treatment and those who discontinued treatment was observed.

## DISCUSSION

Recurrent HCV hepatitis is associated with a significant increase in the morbidity and mortality of transplanted patients due to the early development of graft cirrhosis. In the post-transplant setting, the goals of antiviral treatment are to induce viral eradication and to slow disease progression.

Previous studies<sup>[5,24-27]</sup> have reported biochemical and necro-inflammatory improvement in transplanted patients who achieved a virological response after a course of antiviral treatment; however, the response rate is still unsatisfactory (17%-30% with IFN plus RBV; 18%-45% with PEG-IFN plus RBV)<sup>[5-9]</sup>. Among the non-responders, a significant number of patients will develop of graft cirrhosis and liver-related death in a few years<sup>[2,3]</sup>. To slow disease progression, the efficacy of antiviral maintenance therapy was evaluated in two studies<sup>[17,18]</sup>, which showed preliminary evidence of benefit. Our study aimed to evaluate the efficacy of long-term treatment with In- $\alpha$ -IFN plus ribavirin.

We reported an overall SVR rate of 28.3%; our response rate was similar to those observed with IFN-based and PEG-IFN-based regimens<sup>[5-9]</sup>. We did not observe any different virological outcomes between patients receiving daily and *in vivo* IFN; this result could be due to variation in the HCV genotype distribution. Six patients of 16 (37.5%) in the Group B had a favorable

genotype (2 or 3).

The role of cyclosporine in patients with HCV recurrence is still controversial. Our previous experience has demonstrated that the type of immunosuppression during antiviral treatment may predict the SVR<sup>[28]</sup>, but a meta-analysis failed to demonstrate a significant difference in clinical outcome (graft survival and mortality)<sup>[29]</sup>. Our results showed that a cyclosporine-based immunosuppressive regimen correlated with an increased SVR rate, although there was a small number of CyA-treated patients.

The potential efficacy of pegylated IFN-based antiviral treatments in the transplant setting is limited by poor tolerability and a high rate of adverse events (hematological, autoimmune and rejection) leading to dose reduction and/or therapy discontinuation. Moreover, in our center, we previously experienced several (9 of 44 patients) *de novo* cases of autoimmune hepatitis during PEG-IFN plus ribavirin treatment<sup>[12]</sup>; therefore, in this study, to reduce adverse events and increase patient tolerability, we used a natural IFN-based regimen. As expected, we observed a good safety and tolerability profile; no patient developed autoimmune disease or graft rejection, while only one (2.2%) stopped treatment due to anemia.

Histological analysis from paired liver biopsy samples showed a reduction in necro-inflammatory activity in the patients who cleared HCV-RNA and those who received a long-term course of therapy. As in the non-transplant setting, achievement of complete viral eradication led to an improvement in liver inflammation, biochemically and histologically. Also, we observed a significant decrease in activity scores (from  $7.9 \pm 0.7$  to  $4.7 \pm 0.6$ ;  $P = 0.0001$ ) in the non-responders who received long-term treatment with IFN plus RBV.

To evaluate the anti-viral and anti-inflammatory effects of IFN plus RBV treatment, we tested, on paired liver tissue samples, hepatocyte expression of viral proteins using IHC analysis as previously described by Ballardini *et al.*<sup>[21,22]</sup>. As expected, our results showed that patients who cleared HCV did not have HCV-positive hepatocytes in their liver biopsies, while the non-responders who had interrupted antiviral treatment did not have reduced HCV protein expression. Interestingly, the non-responders who received a long-term course of therapy had a significantly reduced percentage of HCV-positive hepatocytes (median: 45%-0%), leading to a significant reduction in liver inflammation. To exclude sampling error, liver HCV-RNA was quantified in those cases; in all of the liver biopsies, HCV-RNA was detected. We hypothesized that the effect of IFN treatment, even in the non-responders, could reduce liver tissue inflammation by reducing the degree of hepatitis C viral antigen staining<sup>[30]</sup>.

The role of antiviral treatment on disease progression is still debated. Patients who achieve an SVR have been shown to have delayed fibrosis progression<sup>[24]</sup>, while some authors have also observed fibrosis regression<sup>[16]</sup>. A pivotal role for antiviral therapy was demonstrated by a previous study that reported treatment was the only variable to be independently associated with histological

improvement or stabilization among patients with HCV recurrence<sup>[16]</sup>.

In our experience, nine patients who cleared HCV had a significantly reduced staging score on liver biopsy; moreover, a significant percentage (84%) of long-term treatment patients had a histological response despite the lack of viral clearance. Although there was a small number of patients, these results suggest the efficacy of long-term antiviral treatment on disease progression independent of a virological response.

The presence of severe HCV recurrence (histological cirrhosis, cholestatic hepatitis or FCH) is associated with a worse clinical outcome; as in the non-transplant setting<sup>[31,32]</sup>, patients with advanced disease have a reduced SVR rate, due to increased adverse events and therapy discontinuation. Moreover, we found that severe HCV recurrence is the only variable to be independently related to the risk of death. These findings suggest that recurrent HCV hepatitis should be treated at the onset of biochemical and histological signs to improve virological and clinical outcomes.

In conclusion, long-term treatment with In- $\alpha$ -IFN plus ribavirin was able to improve histological staging in SVR patients, slow disease progression in non-responders, and demonstrate a good safety and tolerability profile. These findings suggest the importance of long-term treatment for HCV recurrence; this treatment seems to be able to reduce liver decompensation, graft failure and liver-related death.

## ACKNOWLEDGMENTS

We thank Bologna Liver Transplant Group: Pietro Andreone, Giampaolo Bianchi, Sonia Berardi, Maurizio Biselli, Stefano Brillanti, Matteo Cescon, Alessandro Cucchetti, Giorgio Ercolani, Gian Luca Grazi, Marco Lenzi, Simona Leoni, Francesca Lodato, Maria Cristina Morelli, Fabio Piscaglia, Matteo Ravaioli, Claudia Sama, Gabriella Verucchi, Marco Vivarelli.

## COMMENTS

### Background

Hepatitis C virus (HCV) graft re-infection after liver transplantation is almost universal, leading to accelerated, severe liver disease; moreover, antiviral therapy in this setting is less effective. Only patients who achieve a virological response have an improvement in biochemical and necro-inflammatory activity, while the effect of antiviral treatment on disease progression in non-responders is still controversial.

### Research frontiers

The use of long-term maintenance therapy in transplanted patients who do not achieve a virological response is still debated. Some authors have reported that antiviral maintenance treatment could prevent disease progression, leading to an improvement in long-term survival.

### Innovations and breakthroughs

The results support the concept that long-term antiviral treatment leads to a better histological outcome even in patients who do not achieve viral clearance; therefore, long-term antiviral treatment improves disease progression, leading to a better clinical outcome.

### Applications

New direct antiviral agents are changing the approach to HCV treatment,

including in transplanted patients; however, the management of patients who do not achieve a viral response will be a future clinical challenge. The results supported the safety and tolerability of long-term treatment with interferon and ribavirin in patients who did not respond to therapy. We demonstrated that, even in non-responders, long-term treatment improves clinical and histological outcomes.

### Peer review

As a retrospective clinical study on patients with HCV recurrence after liver transplantation, the results proved the efficacy of long-term antiviral treatment with leukocyte natural  $\alpha$ -interferon and ribavirin on disease progression and showed a good safety and tolerability profile.

## REFERENCES

- 1 **Berenguer M**, López-Labrador FX, Wright TL. Hepatitis C and liver transplantation. *J Hepatol* 2001; **35**: 666-678 [PMID: 11690716 DOI: 10.1016/S0168-8278(01)00179-9]
- 2 **Gane EJ**, Portmann BC, Naoumov NV, Smith HM, Underhill JA, Donaldson PT, Maertens G, Williams R. Long-term outcome of hepatitis C infection after liver transplantation. *N Engl J Med* 1996; **334**: 815-820 [PMID: 8596547 DOI: 10.1056/NEJM199603283341302]
- 3 **Berenguer M**, Ferrell L, Watson J, Prieto M, Kim M, Rayón M, Córdoba J, Herola A, Ascher N, Mir J, Berenguer J, Wright TL. HCV-related fibrosis progression following liver transplantation: increase in recent years. *J Hepatol* 2000; **32**: 673-684 [PMID: 10782918 DOI: 10.1016/S0168-8278(00)80231-7]
- 4 **Roche B**, Samuel D. Hepatitis C virus treatment pre- and post-liver transplantation. *Liver Int* 2012; **32** Suppl 1: 120-128 [PMID: 22212582 DOI: 10.1111/j.1478-3231.2011.02714.x]
- 5 **Samuel D**, Bizollon T, Feray C, Roche B, Ahmed SN, Lemonnier C, Cohard M, Reynes M, Chevallier M, Ducerf C, Baulieux J, Geffner M, Albrecht JK, Bismuth H, Treppe C. Interferon- $\alpha$  2b plus ribavirin in patients with chronic hepatitis C after liver transplantation: a randomized study. *Gastroenterology* 2003; **124**: 642-650 [PMID: 12612903 DOI: 10.1053/gast.2003.50095]
- 6 **Wang CS**, Ko HH, Yoshida EM, Marra CA, Richardson K. Interferon-based combination anti-viral therapy for hepatitis C virus after liver transplantation: a review and quantitative analysis. *Am J Transplant* 2006; **6**: 1586-1599 [PMID: 16827859 DOI: 10.1111/j.1600-6143.2006.01362.x]
- 7 **Berenguer M**. Systematic review of the treatment of established recurrent hepatitis C with pegylated interferon in combination with ribavirin. *J Hepatol* 2008; **49**: 274-287 [PMID: 18571272 DOI: 10.1016/j.jhep.2008.05.002]
- 8 **Xirouchakis E**, Triantos C, Manousou P, Sigalas A, Calvaruso V, Corbani A, Leandro G, Patch D, Burroughs A. Pegylated-interferon and ribavirin in liver transplant candidates and recipients with HCV cirrhosis: systematic review and meta-analysis of prospective controlled studies. *J Viral Hepat* 2008; **15**: 699-709 [PMID: 18673428 DOI: 10.1111/j.1365-2893.2008.01019.x]
- 9 **Lodato F**, Berardi S, Gramenzi A, Mazzella G, Lenzi M, Morelli MC, Tame MR, Piscaglia F, Andreone P, Ballardini G, Bernardi M, Bianchi FB, Biselli M, Bolondi L, Cescon M, Colecchia A, D'Errico A, Del Gaudio M, Ercolani G, Grazi GL, Grigioni W, Lorenzini S, Pinna AD, Ravaioli M, Roda E, Sama C, Vivarelli M. 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**: 450-457 [PMID: 18549463 DOI: 10.1111/j.1365-2036.2008.03761.x]
- 10 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]
- 11 **Toniutto P**, Fabris C, Fumo E, Apollonio L, Caldato M, Avellini C, Minisini R, Pirisi M. Pegylated versus standard interferon- $\alpha$  in antiviral regimens for post-transplant recur-

- rent hepatitis C: Comparison of tolerability and efficacy. *J Gastroenterol Hepatol* 2005; **20**: 577-582 [PMID: 15836706 DOI: 10.1111/j.1440-1746.2005.03795.x]
- 12 **Berardi S**, Lodato F, Gramenzi A, D'Errico A, Lenzi M, Bonfadini A, Morelli MC, Tamè MR, Piscaglia F, Biselli M, Sama C, Mazzella G, Pinna AD, Grazi G, Bernardi M, Andreone P. High incidence of allograft dysfunction in liver transplanted patients treated with pegylated-interferon  $\alpha$ -2b and ribavirin for hepatitis C recurrence: possible de novo autoimmune hepatitis? *Gut* 2007; **56**: 237-242 [PMID: 16798778 DOI: 10.1136/gut.2006.092064]
  - 13 **Guido M**, Burra P. De novo autoimmune hepatitis after liver transplantation. *Semin Liver Dis* 2011; **31**: 71-81 [PMID: 21344352 DOI: 10.1055/s-0031-1272834]
  - 14 **Cotler SJ**, Ganger DR, Kaur S, Rosenblate H, Jakate S, Sullivan DG, Ng KW, Gretch DR, Jensen DM. Daily interferon therapy for hepatitis C virus infection in liver transplant recipients. *Transplantation* 2001; **71**: 261-266 [PMID: 11213071 DOI: 10.1097/00007890-200101270-00017]
  - 15 **Beckebaum S**, Cicinnati VR, Karliova M, Dirsch O, Erim Y, Frilling A, Malago M, Broelsch CE, Treichel U, Gerken G. Daily interferon  $\alpha$ -2B and ribavirin combination therapy for liver transplant patients with chronic hepatitis C infection. *Transplant Proc* 2003; **35**: 2080-2081 [PMID: 14529846 DOI: 10.1016/S0041-1345(03)00681-X]
  - 16 **Carrión JA**, Navasa M, García-Retortillo M, García-Pagan JC, Crespo G, Bruguera M, Bosch J, Forns X. Efficacy of antiviral therapy on hepatitis C recurrence after liver transplantation: a randomized controlled study. *Gastroenterology* 2007; **132**: 1746-1756 [PMID: 17484872 DOI: 10.1053/j.gastro.2007.03.041]
  - 17 **Kornberg A**, Küpper B, Tannapfel A, Bärthel E, Thrum K, Settmacher U. Antiviral maintenance treatment with interferon and ribavirin for recurrent hepatitis C after liver transplantation: pilot study. *J Gastroenterol Hepatol* 2007; **22**: 2135-2142 [PMID: 18031371 DOI: 10.1111/j.1440-1746.2006.04516.x]
  - 18 **Walter T**, Scoazec JY, Guillaud O, Hervieu V, Chevallier P, Boillot O, Dumortier J. Long-term antiviral therapy for recurrent hepatitis C after liver transplantation in nonresponders: biochemical, virological, and histological impact. *Liver Transpl* 2009; **15**: 54-63 [PMID: 19109834 DOI: 10.1002/lt.21652]
  - 19 **Knodell RG**, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, Wollman J. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; **1**: 431-435 [PMID: 7308988 DOI: 10.1002/hep.1840010511]
  - 20 Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology* 1997; **25**: 658-663 [PMID: 9049215 DOI: 10.1002/hep.510250328]
  - 21 **Ballardini G**, Groff P, Giostra F, Francesconi R, Miniero R, Ghetti S, Zauli D, Bianchi FB. Hepatocellular codistribution of c100, c33, c22, and NS5 hepatitis C virus antigens detected by using immunopurified polyclonal spontaneous human antibodies. *Hepatology* 1995; **21**: 730-734 [PMID: 7533125 DOI: 10.1002/hep.1840210320]
  - 22 **Ballardini G**, Groff P, Pontisso P, Giostra F, Francesconi R, Lenzi M, Zauli D, Alberti A, Bianchi FB. Hepatitis C virus (HCV) genotype, tissue HCV antigens, hepatocellular expression of HLA-A,B,C, and intercellular adhesion-1 molecules. Clues to pathogenesis of hepatocellular damage and response to interferon treatment in patients with chronic hepatitis C. *J Clin Invest* 1995; **95**: 2067-2075 [PMID: 7738174 DOI: 10.1172/JCI117893]
  - 23 **Grassi A**, Quarneri C, Ravaioli M, Bianchini F, Susca M, D'Errico A, Piscaglia F, Tamè MR, Andreone P, Grazi G, Galli S, Zauli D, Pinna AD, Bianchi FB, Ballardini G. Detection of HCV antigens in liver graft: relevance to the management of recurrent post-liver transplant hepatitis C. *Liver Transpl* 2006; **12**: 1673-1681 [PMID: 17031825 DOI: 10.1002/lt.20882]
  - 24 **Bizollon T**, Ahmed SN, Radenne S, Chevallier M, Chevallier P, Parvaz P, Guichard S, Ducerf C, Baulieux J, Zoulim F, Trepo C. Long term histological improvement and clearance of intrahepatic hepatitis C virus RNA following sustained response to interferon-ribavirin combination therapy in liver transplanted patients with hepatitis C virus recurrence. *Gut* 2003; **52**: 283-287 [PMID: 12524414 DOI: 10.1136/gut.52.2.283]
  - 25 **Roche B**, Sebah M, Canfora ML, Antonini T, Roque-Afonso AM, Delvart V, Saliba F, Duclos-Vallee JC, Castaing D, Samuel D. Hepatitis C virus therapy in liver transplant recipients: response predictors, effect on fibrosis progression, and importance of the initial stage of fibrosis. *Liver Transpl* 2008; **14**: 1766-1777 [PMID: 19025933 DOI: 10.1002/lt.21635]
  - 26 **Bizollon T**, Pradat P, Mabrut JY, Chevallier M, Adham M, Radenne S, Souquet JC, Ducerf C, Baulieux J, Zoulim F, Trepo C. Benefit of sustained virological response to combination therapy on graft survival of liver transplanted patients with recurrent chronic hepatitis C. *Am J Transplant* 2005; **5**: 1909-1913 [PMID: 15996238 DOI: 10.1111/j.1600-6143.2005.00976.x]
  - 27 **Abdelmalek MF**, Firpi RJ, Soldevila-Pico C, Reed AI, Hemming AW, Liu C, Crawford JM, Davis GL, Nelson DR. Sustained viral response to interferon and ribavirin in liver transplant recipients with recurrent hepatitis C. *Liver Transpl* 2004; **10**: 199-207 [PMID: 14762857 DOI: 10.1002/lt.20074]
  - 28 **Cesccon M**, Grazi GL, Cucchetti A, Vetrone G, Ravaioli M, Ercolani G, Morelli MC, Piscaglia F, Tamè M, Pinna AD. Predictors of sustained virological response after antiviral treatment for hepatitis C recurrence following liver transplantation. *Liver Transpl* 2009; **15**: 782-789 [PMID: 19562715 DOI: 10.1002/lt.21760]
  - 29 **Berenguer M**, Royuela A, Zamora J. Immunosuppression with calcineurin inhibitors with respect to the outcome of HCV recurrence after liver transplantation: results of a meta-analysis. *Liver Transpl* 2007; **13**: 21-29 [PMID: 17192906 DOI: 10.1002/lt.21035]
  - 30 **Di Bisceglie AM**, Hoofnagle JH, Krawczynski K. Changes in hepatitis C virus antigen in liver with antiviral therapy. *Gastroenterology* 1993; **105**: 858-862 [PMID: 7689520]
  - 31 **Bruno S**, Shiffman ML, Roberts SK, Gane EJ, Messinger D, Hadziyannis SJ, Marcellin P. Efficacy and safety of peginterferon alfa-2a (40KD) plus ribavirin in hepatitis C patients with advanced fibrosis and cirrhosis. *Hepatology* 2010; **51**: 388-397 [PMID: 19918980 DOI: 10.1002/hep.23340]
  - 32 **Cheng WS**, Roberts SK, McCaughan G, Sievert W, Weltman M, Crawford D, Rawlinson W, Marks PS, Thommes J, Rizkalla B, Yoshihara M, Dore GJ. Low virological response and high relapse rates in hepatitis C genotype 1 patients with advanced fibrosis despite adequate therapeutic dosing. *J Hepatol* 2010; **53**: 616-623 [PMID: 20619475 DOI: 10.1016/j.jhep.2010.04.024]

P- Reviewers Lu XM, Yu B

S- Editor Zhai HH L- Editor A E- Editor Zhang DN







百世登

**Baishideng**®

Published by **Baishideng Publishing Group Co., Limited**

Flat C, 23/F., Lucky Plaza,

315-321 Lockhart Road, Wan Chai, Hong Kong, China

Fax: +852-65557188

Telephone: +852-31779906

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

<http://www.wjgnet.com>



ISSN 1007-9327



9 771007 932045