Digestive and Liver Disease 44 (2012) 861–867

Contents lists available at SciVerse ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Liver, Pancreas and Biliary Tract

Long-term antiviral treatment for recurrent hepatitis C after liver transplantation

Valentina Rosa Bertuzzo[a], Matteo Cescon[a,*], Maria Cristina Morelli[a], Paolo Di Gioia[a], Mariarosa Tamè[b], Stefania Lorenzini[b], Pietro Andreone[b], Giorgio Ercolani[a], Massimo Del Gaudio[a], Matteo Ravaioli[a], Alessandro Cucchetti[a], Alessandro Dazzi[a], Antonietta D'Errico-Grigioni[c], Antonio Daniele Pinna[a]

[a] Department of General Surgery and Organ Transplantation, University of Bologna, Bologna, Italy
[b] Department of Digestive Diseases and Internal Medicine, University of Bologna, Bologna, Italy
[c] Department of Oncology, Haematology and Laboratory Medicine, University of Bologna, Bologna, Italy

ARTICLE INFO

Article history:
Received 16 December 2011
Accepted 13 June 2012
Available online 21 July 2012

Keywords:
Antiviral treatment
HCV recurrence
Liver transplantation
Non-responder

ABSTRACT

Background and aims: The management of patients treated for hepatitis C recurrence after liver transplantation and not achieving virological response following treatment with interferon plus ribavirin is controversial.
Methods: A retrospective analysis of the outcomes of 70 patients non-responders to antiviral treatment after liver transplantation was performed. Twenty-one patients (30.0%; Group A) were treated for ≤12 months and 49 (70.0%; Group B) for more than 12 months.
Results: The 2 groups were comparable for main demographic, clinical and pathological variables. Median duration of antiviral treatment was 8.2 months in Group A and 33.4 months in Group B. No patient achieved a complete virological response. The 5-year patient hepatitis C-related survival rate was 49.2% in Group A and 88.3% in Group B ($P = 0.002$), while the 5-year graft survival rate was 49.2% in Group A and 85.9% in Group B ($P = 0.007$). The median yearly fibrosis progression rate was 1.21 per year in Group A and 0.40 per year in Group B ($P = 0.001$).
Conclusions: Prolonged antiviral treatment showed an overall beneficial effect in transplanted patients with a recurrent hepatitis C infection and not responding to conventional therapy. The treatment should be continued as long as it is permitted, in order to improve clinical and histological outcomes.
© 2012 Editrice Gastroenterologica Italiana S.r.l. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Hepatitis C virus (HCV) infection is the major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma in most developed countries and it is the most frequent indication for orthotopic liver transplantation (OLT) in Europe and in the United States [1,2].

Although OLT is an effective treatment to reduce morbidity and mortality in this population, almost all recipients develop recurrent infection of the graft; the principal factor related to a more severe HCV recurrence is advanced donor age [3–5]. Because of immunosuppression, histological progression of HCV infection is more rapid than in non-transplanted patients, with 5-year cirrhosis progression between 20 and 40% [6]. Subsequently, HCV patients have a poorer prognosis after OLT compared to those with other indications [7].

Treatment options for HCV recurrence after OLT include antiviral treatment (AVT), based on a combination of standard interferon (IFN) or pegylated interferon (PEG-IFN) plus ribavirin (RBV), or re-transplantation (re-OLT). re-OLT is reserved for a small percentage of patients [8–10] when virological response (VR) is not achieved and decompensated graft cirrhosis has been established; AVT is recommended worldwide when histological evidence of recurrent hepatitis C is documented [11]. Unfortunately, a sustained virological response (SVR) is achieved in only 20% to 40% of patients treated with AVT [12,13]. Factors related to a lower probability of VR include high pre-treatment viral load, genotype 1, absence of early virological response and the administration of antiviral therapy at a reduced dosage due to side effects [8,13–16].

Although it has been recognized that IFN plus RBV treatment is crucial for HCV recurrence prognosis after OLT, no standard strategy has yet been established [13–16]; in particular, patient selection, timing of initiation, dosage schedule and duration are still controversial issues.

In addition, little is known about the use of long-term maintenance therapy in transplanted patients without VR [17–22]. Walter et al. [21] reported a decreased fibrosis progression in patients

* Corresponding author at: Unità Operativa Chirurgia Generale e Trapianti, Padiglione 25, Policlinico Sant'Orsola-Malpighi, Via Massarenti 9, 40138 Bologna, Italy. Tel.: +39 051 6364810; fax: +39 051 304902.
E-mail address: matteo.cescon@aosp.bo.it (M. Cescon).

1590-8658/$36.00 © 2012 Editrice Gastroenterologica Italiana S.r.l. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.dld.2012.06.013

treated for more than 6 months, even in the absence of VR. Conversely, Ikegami et al. [22] described a stabilization in fibrosis stage, regardless of AVT duration, in VR patients without SVR and in non-responders (NR) with biochemical response (BR), but a worsening in fibrosis stage in patients who showed neither BR nor VR to AVT.

The aim of our study was therefore to evaluate the effect of prolonged AVT in NR, with particular consideration on patient survival and fibrosis progression rate.

2. Materials and methods

Between January 1st, 2000 and December 31st, 2009, 126 HCV-positive transplanted patients received AVT due to post-transplant HCV recurrence at our outpatient clinic; 88 of them (69.8%) did not achieve a complete VR. Among them, we excluded 2 patients (2.3%) because we lost them to follow-up, and 16 patients (18.2%) with early cholestatic recurrence and a rapidly progressive course, in whom the AVT was performed for a very short time. All these 16 patients died due to HCV recurrence within 12 months after transplant. Hence, study population consisted of 70 patients. Three (4.3%) patients underwent re-OLT for graft cirrhosis due to HCV recurrence. For one patient receiving AVT only before re-OLT, the follow-up was censored at the date of re-OLT, while for one patient in whom AVT was administered only after re-OLT, all the data were collected starting from this point. For the patient treated after both transplantations, the starting point was considered the first OLT.

HCV infection was defined as positivity for serum anti-HCV antibodies, while hepatitis B virus (HBV) infection was defined as positivity of hepatitis B surface antigen (HBsAg) or of anti-core antibodies (HBcAb) at the time of surgery. Human immunodeficiency virus (HIV) was defined as positivity for serum anti-HIV antibodies.

Severity of liver dysfunction was graded according to the Model for End-stage Liver Disease (MELD) score currently used by UNOS (http://www.unos.org) [23].

2.1. HCV detection and genotyping

Quantitative serum HCV-RNA was routinely determined in all patients with a branched DNA assay (Quantiplex HCV 2.0, Chiron Corp). The lowest limit of detection of the quantitative assay was 0.615×10^3 IU/mL, while the highest one was 7.692×10^6 IU/mL. Viral genotype was determined by nested reverse transcription polymerase chain reaction of the core region with type-specific primers (Inno-LiPA HCV, Innogenetics, Ghent, Belgium) and classified according to Simmonds criteria [24].

2.2. Diagnosis of hepatitis C recurrence

Three criteria had to be fulfilled to diagnose recurrent hepatitis C: (1) alteration of liver function tests in the absence of vascular, biliary, drug, or infectious causes; (2) liver biopsy confirming HCV recurrence; (3) detectable quantitative HCV-RNA in the serum. Histological staging and grading of chronic HCV-related graft hepatitis were performed according to the Ishak scoring system [25].

No routine biopsies were usually performed at our Centre. Liver biopsy samples were obtained before starting AVT and when clinically indicated. Disease progression/regression was assessed by computing the yearly fibrosis progression rate (yFPR), which was obtained dividing the absolute change in the fibrosis score by the years of observation [26].

2.3. Immunosuppression

Cyclosporine A (CyA) and tacrolimus (TAC) were the main immunosuppressive drugs used in this study population, both associated with steroids. The assignment was not dictated by a specific choice but simply reflected the increasing use of TAC as the primary immunosuppressive agent by most programmes during the study period. m-TOR inhibitors (mammalian Target Of Rapamycin inhibitors; sirolimus and everolimus) were either administrated as primary immunosuppressive agents or in combination with reduced doses of calcineurin inhibitors in the event of side-effects. During AVT, serum levels of CyA were maintained between 90 and 150 ng/mL, those of TAC were maintained between 4 and 10 ng/mL, and those of sirolimus and everolimus were maintained between 3 and 8 ng/mL.

Anti-CD25 antibodies (basiliximab or daclizumab) or anti-thymocyte globulins were used at the time of transplantation as induction therapy in a minority of patients.

2.4. Antiviral therapy

No patients received pre-emptive AVT. The minimum duration of AVT was 6 months, regardless of the achievement of a complete virological and biochemical response during this period and unless adverse events contraindicating AVT occurred. After 2002, an attempt to treat patients with genotypes 1 and 4 for 12 months was routinely made.

General criteria for dose reduction/discontinuation are those reported below, and they were fulfilled in all patients. No patient voluntarily stopped AVT in the absence of clinical indications. AVT was avoided or discontinued in patients who developed severe rejection, systemic bacterial infection, symptomatic anaemia, or severe depression despite antidepressants. The response to AVT was defined based on virological and biochemical outcomes. VR was defined as negative serum HCV-RNA during AVT; BR was defined as a serum Alanine Transaminase (ALT) level that decreased to and remained in the normal range (ALT < 31 IU/L) during the treatment for at least 3 months.

AVT was started with 1.5 MU of IFN α-2b 3 times weekly plus 400–600 mg of RBV daily for 1–2 weeks, and if well tolerated, doses were increased to 3 MU of IFN α-2b 3 times weekly plus up to 1200 mg of RBV daily. In 2002, this regimen was replaced by 135–180 μg of PEG-IFN α-2a or 50–80 μg of PEG-IFN α-2b weekly plus weight-adjusted daily RBV. Some patients who initially did not respond to IFN α-2b were subsequently switched to PEG-IFN.

Granulocyte colony stimulating factor was used when the neutrophil count was lower than 800 cells/μL. IFN doses were reduced when the neutrophil count was lower than 800 cells/μL and/or the platelet count was lower than 50,000 μL^{-1}. IFN was stopped when the neutrophil count was lower than 500 cells/μL and/or the platelet count was lower than 20,000 μL^{-1}.

Erythropoietin alpha was administered when the haemoglobin level was lower than 10 g/dL. RBV dose reduction was considered when the haemoglobin level was lower than 10 g/dL despite erythropoietin therapy, and it was stopped when the haemoglobin level was lower than 8 g/dL.

2.5. Statistical analysis

Results were expressed as median and range of values. Differences between continuous and categorical variables were calculated with the Mann–Whitney *U* test and the χ^2-test or Fisher's exact test, respectively.

HCV-related survival (HCV-S) was computed from the day of surgery or of starting of AVT to the day of death due to HCV recurrence or the last follow-up visit. Patients who died due to causes other than HCV recurrence were censored at the date of death. We conducted the analysis according to the Kaplan–Meier method and compared the differences between groups by the log-rank test. Logistic regression was used for multivariate analysis of risk factors for lower survival.

Table 1
Demographic, clinical and pathological parameters of 70 transplanted patients with recurrent hepatitis C virus infection which not responded to antiviral treatment at the Bologna Centre between 2000 and 2009.

Variables	
Recipient gender	
Male	51 (72.9%)
Female	19 (27.1%)
Recipient age at OLT (years)	58 (36–68)
Recipient BMI at OLT (kg/m^2)	24.9 (17.2–34.5)
Recipient MELD score at OLT	18 (7–37)
Recipient genotype	
1	58 (82.9%)
Others	12 (17.1%)
Recipient HBV co-infection	5 (7.1%)
Recipient anti-HIV positivity	4 (5.7%)
Donor gender	
Male	41 (59.1%)
Female	29 (40.9%)
Donor age (years)	68 (14–88)
Donor anti-HCV positivity	10 (14.3%)
Fibrosis score in anti-HCV positive graft biopsies	1 (0–1)
Donor anti-HBc positivity	10 (14.3%)
CIT (min)	380 (150–673)
Induction therapy at OLT	11 (15.7%)
Immunosuppressive drugs	
TAC	53 (75.7%)
CyA	11 (15.7%)
m-TOR inhibitors	6 (8.6%)
MMF	1 (1.4%)
Steroids	61 (87.1%)
Time between OLT and HCV recurrence (months)	4.0 (0.3–147.0)
Delay between HCV recurrence and AVT start (months)	0.7 (0–20.5)
HCV-RNA level before AVT ($\times 10^6$ IU/mL)	2.614 (0.128–7.692)
HCV-RNA level after 3 months of AVT ($\times 10^6$ IU/mL)	1.994 (0.110–7.692)
HCV-RNA level at the EOT or last follow-up ($\times 10^6$ IU/mL)	0.451 (0.008–7.692)
Fibrosis score before AVT	2 (0–5)
Fibrosis score at the EOT or last histology	3 (1–5)
Necroinflammation score before AVT	6 (1–16)
Necroinflammation score at the EOT or last histology	6 (1–11)
Type of IFN administered	
IFN α-2b	50 (71.4%)
PEG-IFN[a]	32 (45.7%)
Duration of AVT (months)	19.1 (3.3–84.6)
Autoimmune hepatitis during AVT	1 (1.4%)
Biochemical response	44 (62.9%)
Dose reduction	19 (27.1%)
AVT temporary suspension	14 (20.0%)
yFPR (U/year)	0.48 (−1.54 to 6.97)

Data are expressed as median (range) or number (%).
OLT, orthotopic liver transplantation; BMI, body mass index; MELD, model for end-stage liver disease; HBV, hepatitis B virus; HIV, human immunodeficiency virus; HCV, hepatitis C virus; anti-HBc, antibodies anti-hepatitis B core antigen; CIT, cold ischaemia time; TAC, tacrolimus; CyA, cyclosporine A; m-TOR, mammalian target of rapamycin; MMF, mycophenolate mofetil; AVT, antiviral treatment; EOT, end of treatment; IFN, interferon; PEG, pegylated; yFPR, yearly fibrosis progression rate.
[a] Included patients treated before with IFN α-2b.

Considering a 30% decrease in HCV-S between patients with long-term AVT and those with short term AVT, with a 5% type I error and 80% statistical power for a one-tailed log-rank test, the population size of the 2 groups of patients is 33.

A P value <0.05 was considered statistically significant. Statistical analysis was carried out with the SPSS software packaging (SPSS Inc., Chicago, IL, USA), version 13.

3. Results

3.1. Characteristics of the study population

Patient characteristics are reported in Table 1. The median duration of AVT was 19.1 months (range: 3.3–84.6). Twelve patients (17.1%) who initially did not respond to IFN α-2b were subsequently switched to PEG-IFN. BR was achieved in the majority of patients (62.9%); in contrast, no patient achieved VR, but median HCV-RNA level fell from 2.614×10^6 IU/mL before AVT to 0.451×10^6 IU/mL at the end of treatment or at the last follow-up.

The median time between beginning of AVT and the last control liver biopsy was 10.6 months (range: 1.8–54.4). According to the Ishak scoring system, the fibrosis stage before AVT was 0–3 in 61 patients (87.2%) and 4–6 in 9 patients (14.8%). Fibrosis stabilization or improvement, defined as yearly fibrosis progression rate (yFPR) ≤0 was found in 41.4% of patients, while in 58.6% a worsening occurred.

Overall discontinuation rate was 58.6%. AVT intolerance/toxicity and absence of response were the main causes of treatment discontinuation (31.7% and 48.8%, respectively). Dose reduction and temporary interruption of AVT due to side effects were needed in 19 patients (27.1%) and 14 (20.0%), respectively.

None of our patients developed acute/chronic rejection during AVT. Autoimmune hepatitis was observed in one patient (1.4%); in this case AVT was interrupted.

3.2. Comparison between standard and prolonged AVT

The study population was divided into two groups, according to the duration of AVT: 21 patients (30.0%; Group A) were treated for ≤12 months and 49 patients (70.0%; Group B) for more than 12 months.

Median duration of AVT was 8.2 months (range: 3.3–11.7) in Group A and 33.4 months (range: 12.1–84.6) in Group B.

With the exception of a higher prevalence of HCV-positive donors in Group B, the 2 groups were comparable for demographic, clinical, histological, donor-related, viral-related and AVT-related variables (Table 2).

In particular, the median time between OLT and HCV recurrence was 4.4 months in Group A (range: 0.3–34.7 months) and 4.2 months in Group B (range: 0.3–147.0 months) ($P = 0.135$), while the median delay between HCV recurrence diagnosis and AVT starting was 0.7 months in Group A (range: 0–4.7 months) and 0.7 months in Group B (range: 0–20.5 months) ($P = 0.229$).

Median HCV-RNA in Group A and B was comparable before AVT (Group A: 2.308×10^6 IU/mL; range: $0.537–7.692 \times 10^6$ IU/mL; Group B: 2.808×10^6 IU/mL; range: $0.128–7.692 \times 10^6$ IU/mL; $P = 0.600$) and at the end of treatment/last follow-up (Group A: 0.789×10^6 IU/mL; range: $0.008–7.692 \times 10^6$ IU/mL; Group B: 0.397×10^6 IU/mL; range: $0.016–7.692 \times 10^6$ IU/mL; $P = 0.355$).

Although not reaching the threshold of significance, BR showed a trend towards higher rates in Group B ($P = 0.073$). Toxicity-related criteria for AVT suspension were the same in the 2 study groups. Causes of AVT discontinuation are reported in Table 3. AVT intolerance/toxicity and absence of response were the main causes of AVT discontinuation in both groups (90.5% and 70.0%, respectively) Median time to discontinuation due to AVT intolerance/toxicity was 7.0 months in Group A (range: 3.3–11.6) and 29.9 months (range: 15.0–35.8) in Group B ($P = 0.001$).

3.3. Evaluation of fibrosis progression

The median time between beginning of AVT and control liver biopsy was 10.4 months in Group A (range: 3.0–54.4 months) and 11.9 months in Group B (range: 1.8–47.7 months) ($P = 0.201$).

Even if hepatitis staging score before and at the end of AVT did not reach the threshold of significance, the median yFPR was 1.21 per year in Group A (range: 0–6.97 per year) and 0.40 per year in Group B (range: −1.54 to 2.31 per year) ($P = 0.001$). Fibrosis stabilization/regression and fibrosis worsening were found in 14.3% and

Table 2
Comparison of patients characteristics in standard (Group A) and prolonged (Group B) antiviral treatment groups.

Variables	Group A (21 patients)	Group B (49 patients)	*P*
Recipient gender (M/F)	15/6 (71.4/28.6%)	36/13 (73.5/26.5%)	0.539
Recipient age > 60 years	5 (23.8%)	17 (34.7%)	0.272
Recipient BMI > 25 kg/m^2	8 (38.1%)	15 (30.6%)	0.575
Recipient genotype 1	18 (85.7%)	40 (81.6%)	0.160
Recipient genotype other than 1	3 (14.3%)	9 (18.4%)	0.236
Recipient HBV co-infection	2 (9.5%)	3 (6.1%)	0.475
Recipient HIV positivity	2 (9.5%)	2 (4.1%)	0.347
MELD score > 20	7 (33.3%)	21 (42.9%)	0.204
Donor gender (M/F)	16/5 (76.2/23.8%)	25/24 (51.1/48.9%)	0.065
Donor age > 60 years	12 (57.1%)	28 (57.1%)	0.489
Donor HCV positivity	0	10 (20.4%)	0.018
Donor anti-HBc positivity	4 (19.0%)	6 (12.2%)	0.385
CIT > 8 h	5 (23.8%)	10 (20.4%)	0.578
Fibrosis score before AVT	1 (0–4)	2 (0–5)	0.139
Fibrosis score at the EOT or last histology	3 (1–4)	3 (1–5)	0.673
Necroinflammation score before AVT	6 (4–10)	6 (1–16)	0.563
Necroinflammation score at the EOT or last histology	6 (5–8)	6 (1–11)	0.727
Fibrosis score 4–6 before AVT	1 (6.3%)	8 (16.3%)	0.280
Fibrosis score 4–6 at the EOT or last histology	3 (14.3%)	11 (22.4%)	0.302
Induction therapy at OLT	3 (14.3%)	8 (16.3%)	0.570
TAC administration	15 (71.4%)	38 (77.6%)	0.468
CyA administration	5 (23.8%)	6 (12.2%)	0.193
m-TOR inhibitor administration	1 (6.3%)	5 (10.2%)	0.412
MMF administration	1 (6.3%)	0	0.300
Steroids administration	21 (100%)	40 (81.6%)	0.095
Time between OLT and HCV recurrence (months)	4.4 (0.3–34.7)	4.2 (0.3–147.0)	0.135
Delay between HCV recurrence and AVT start (months)	0.7 (0–4.7)	0.7 (0–20.5)	0.229
HCV-RNA level before AVT ($\times 10^6$ IU/mL)	2.308 (0.537–7.692)	2.808 (0.128–7.692)	0.600
HCV-RNA level after 3 months of AVT ($\times 10^6$ IU/mL)	1.988 (0.436–7.692)	2.001 (0.110–7.692)	0.956
HCV-RNA level at the EOT or last follow-up ($\times 10^6$ IU/mL)	0.789 (0.008–7.692)	0.397 (0.016–7692)	0.355
Type of IFN (IFN α-2b/PEG-IFN*)	9/12 (42.9%/57.1%)	29/20 (59.2%/40.8%)	0.160
Autoimmune hepatitis during AVT	1 (6.3%)	0	0.300
Biochemical response	10 (47.6%)	34 (69.4%)	0.073
Dose reduction	6 (28.6%)	13 (26.5%)	0.539
AVT temporary suspension	3 (14.3%)	11 (22.4%)	0.333
yFPR (U/year)	1.21 (0–6.97)	0.40 (−1.54–2.31)	0.001

Data are expressed as median (range) or number (%).
M/F, male/female; BMI, body mass index; HBV, hepatitis B virus; HIV, human immunodeficiency virus; MELD, model for end-stage liver disease; HCV, hepatitis C virus; anti-HBc, antibodies anti-hepatitis B core antigen; CIT, cold ischaemia time; AVT, antiviral treatment; EOT, end of treatment; TAC, tacrolimus; CyA, cyclosporine A; m-TOR, mammalian target of rapamycin; MMF, mycophenolate mofetil; OLT, orthotopic liver transplantation; IFN, interferon; PEG, pegylated; yFPR, yearly fibrosis progression rate.
* Included patients treated before with IFN α-2b.

85.7% of patients in Group A and in 45.9% and 54.1% of patients in Group B, respectively ($P = 0.125$).

Although patients with BR were more likely to have fibrosis stabilization/regression, the correlation between these 2 variables was not statistically significant ($P = 0.395$) (Fig. 1).

3.4. Analysis of survival

Median follow-up after OLT was 54.7 months (range: 7.1–205.3). Median follow-up was 34.6 months in Group A (range: 7.1–144.1 months) and 57.6 months in Group B (range: 14.2–205.3 months) ($P = 0.229$).

Table 3
Comparison of causes of treatment discontinuation in standard (Group A) and prolonged (Group B) antiviral treatment groups.

Causes of AVT discontinuation	Group A (21/21 patients)	Group B (20/49 patients)
AVT intolerance/toxicity	9 (42.9%)	4 (20.0%)
Absence of response	10 (47.6%)	10 (50.0%)
Hepatic failure	0	2 (10.0%)
Death	2 (9.5%)	4 (20.0%)

Data are expressed as number (%).
AVT, antiviral treatment.

At the end of the follow-up period, 51 (72.9%) patients were alive and 19 (27.1%) had died. The causes of death were HCV recurrence in 17 (89.5%) cases, other infections in one (5.3%) case, and occurrence of neurologic complications in one (5.3%) case. Five-year overall survival rate was 74.7%.

Graft loss due to HCV recurrence occurred in 17 patients; 5-year HCV-related survival rate (HCV-S) was 76.4%. Five-year HCV-S after beginning of AVT was 68.7%, while 5-year overall survival rate after starting of AVT was 67.6%.

Five-year patient HCV-S rate was 49.2% in Group A and 88.3% in Group B ($P = 0.002$), while 5-year graft survival rate was 49.2% in Group A and 85.9% in Group B ($P = 0.007$).

Five-year patient HCV-S rate after beginning of AVT was 45.6% in Group A and 76.4% in Group B ($P = 0.001$). Five-year graft survival rate after beginning of AVT was 45.6% in Group A and 74.8% in Group B ($P = 0.007$).

The univariate and multivariate analyses of factors affecting survivals are summarized in Table 4. Among all demographic, clinical, histological, donor-related, viral-related and AVT-related variables depicted in Table 2, only AVT duration >12 months, occurrence of BR and absence of AVT temporary discontinuation were predictors of higher overall survival and/or HCV-S.

Considering only patients infected by genotype 1 HCV, all the above analyses (comparison of study groups, evaluation of fibrosis progression and survival analysis) demonstrated identical results (data not shown).

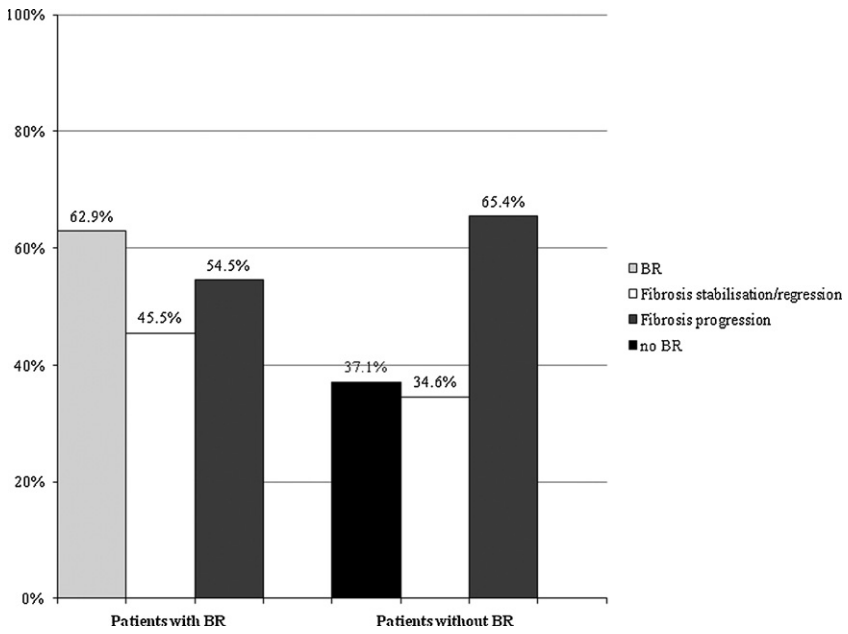

Fig. 1. Comparison of fibrosis stabilisation/regression rate and fibrosis progression rate in patients with or without biochemical response ($P = 0.395$). BR, biochemical response.

4. Discussion

Hepatitis C recurrence after liver transplantation is one of the most important problems of transplant programmes. Liver injury caused by HCV recurrence is accelerated, and cirrhosis develops in a significant proportion of patients only a few years after transplantation [3–7]. Most centres recommend AVT once a liver biopsy specimen demonstrates the presence of significant histological damage [11].

Unfortunately, a SVR may be obtained in only 20–40% of treated patients [12,13], especially due to poor tolerance to AVT of transplant recipients [16]; another important reason could be the slower rate of virological clearance observed in this subset of patients vs. the non-transplant population, which is likely to be multifactorial, including the consequences of immunosuppression, AVT suspension or dose reduction [27].

To our knowledge, our study is the first in the published literature in which only NR patients were considered. All the patients who died due to cholestatic recurrence during AVT administered for less than 12 months were excluded to avoid a possible bias. According to AVT duration (≤12 and >12 months), we retrospectively divided the study population into two groups. These groups were homogeneous in characteristics; in fact, only donor HCV serology reached the threshold of significance ($P = 0.018$), which is thought to be a coincidence. In any case, it has been confirmed that anti-HCV positivity of donors does not represent a significant risk factor for post-transplant HCV recurrence and graft and patient survival provided that no or minimal fibrosis is present in the graft [28–30]. The decision to transplant an organ from an anti-HCV positive donor was uniformly taken throughout the study period when the graft looked macroscopically normal, and frozen section histology at the time of harvesting showed only minimal to mild inflammation, and no to minimal fibrosis.

It is important to note that the need for dose reduction or AVT temporary suspension due to side effects was similar in the two groups.

We found that the use of AVT over the standard duration was associated with a significant increase of both 5-year HCV-S rate and 5-year graft survival rate. Furthermore, the median yFPR was significantly slower in Group B (0.40 per year) compared to Group A (1.21 per year). Although a yFPR of 0.48 per year was used to define patients with an accelerated pattern of fibrosis progression [26], we must consider that our population consisted exclusively of NR, and that no patient finally achieved a VR. Despite the lack of

Table 4
Univariate and multivariate analysis of factors significantly affecting survival in non-responder patients treated with antiviral treatment for hepatitis C recurrence after liver transplantation.

	Variable	Univariate analysis			Multivariate analysis		
		Category	5-Year survival	*P* value	Exp (B)	95% C.I.	*P* value
OS	AVT duration	>12 months vs. ≤12 months	85.9% vs. 49.2%	0.007	3.027	1.218–7.523	0.017
	BR occurrence	Yes vs. no	94.6% vs. 42.8%	<0.0001	8.763	2.879–26.670	<0.0001
HCV-S	AVT duration	>12 months vs. ≤12 months	88.3% vs. 49.2%	0.002	6.230	2.073–18.721	0.001
	BR occurrence	Yes vs. no	97.4% vs. 42.8%	<0.0001	20.755	4.437–97.094	<0.0001
	AVT temporary suspension	Yes vs. no	58.4% vs. 80.3%	0.029	0.178	0.055–0.579	0.004
OS from AVT	AVT duration	>12 months vs. ≤12 months	74.8% vs. 45.6%	0.007	2.975	1.185–7.467	0.02
	BR occurrence	Yes vs. no	87.8% vs. 35.8%	<0.0001	8.330	2.726–25.452	<0.0001
HCV-S after AVT	AVT duration	>12 months vs. ≤12 months	76.4% vs. 45.6%	0.001	6.717	2.221–20.312	0.001
	BR occurrence	Yes vs. no	90.0% vs. 35.8%	<0.0001	19.778	4.209–92.944	<0.0001
	AVT temporary suspension	Yes vs. no	27.8% vs. 76.4%	0.028	0.191	0.06–0.613	0.005

OS, overall survival; AVT, antiviral treatment; BR, biochemical response; HCV-S, hepatitis C related survival.

statistical significance, we found a tendency to fibrosis stabilization or improvement in Group B compared to Group A. Considering the similar follow-up of the two groups, we gathered that the difference in median yFPR reflected the prolongation of AVT.

The number of patients included in Group A was slightly lower than that required by a sample size calculation based on the different survivals observed between groups. However, univariate and multivariate analyses confirmed that the use of AVT over the standard duration and BR were independently associated with a significant increase of patient and graft survival rates. Although BR was a predictor of longer survival, its correlation with fibrosis progression was not as relevant as that of AVT duration.

Data assessing changes in liver histology following AVT in HCV-infected transplant recipients are limited and the results of several series are controversial [12,21,22,31,32]. In fact, while it was demonstrated that SVR was the only independent predictor of histological response in treated patients [12], other authors proved that the stabilization or the regression of fibrosis was observed in 42% of treated patients without a SVR [21]. In contrast, Berenguer et al. found no statistically significant changes in histological findings between pre-therapy and post-treatment liver biopsies, either in patients who achieved SVR or in NR [33]. Further, Neumann and colleagues [33] reported that the fibrosis progression rate showed a rapid and exponential increase during the first 3 years in recipients with recurrent HCV infection. Correlating these data to AVT initiation, it becomes evident that the earlier AVT is started the better the chances to delay fibrosis progression.

In our experience, although the median duration of AVT was 19 months, none of our patients achieved VR. Walter et al. reported a VR rate of 11.5% at 3 months, 28.6% at 6 months and 37% at 12 months [21]. In NR patients, they obtained a further VR rate of 44% at 18 months and 48.5% at the end of the follow-up [21]. Possible explanations of the difference between our data and those reported by Walter are that our patients were more frequently infected with genotype 1 (82.9% vs. 72%), had a higher median pre-treatment viral load (2.6×10^6 vs. 1.2×10^6 IU/mL) and had received grafts from older donors (68 vs. 35 years). All the above factors are well known indicators of a low probability of achieving VR [3–5,8,13–16].

Nevertheless, the median HCV-RNA level (IU/mL) fell from more than 2 million before AVT initiation to less than half a million at the end of treatment or at the last follow-up.

Although our study is limited by its retrospective nature and the small number of patients included, these results support the concept that, in transplant patients affected by HCV recurrence, AVT should be continued as long as it is permitted by clinical and laboratory data, in order to control viral replication and, potentially, to allow disease stabilization or improvement.

The evidence of benefits of protracting AVT in spite of persistent HCV-RNA positivity, with careful management of toxicity events, did increase during our experience. The parallel observation of rapid deterioration of liver function immediately after stopping AVT in most non-responders reinforced our policy of AVT prolongation in this population. After 2003, AVT had to be interrupted within 12 months only in 3 non-responders without side effects due to a negative predisposition to receive INF in front of the absence of virological response.

Our data also revealed that long-term AVT is safe, with only one postoperative death due to infection possibly attributable to AVT administration. In this view, one could speculate on the opportunity of the prolonged use of standard IFN instead of switching these patients to PEG-IFN, because the second drug is certainly more effective but probably carries a high risk of side effects in the long term.

In conclusion, our results supported the feasibility of long-term AVT in transplanted patients with a recurrent HCV infection who did not respond to conventional therapy. Even in the absence of VR, the treatment could be continued as long as it is permitted, with the aim of improving the clinical and histological outcomes.

Prospective studies that integrate prolonged AVT and systematic histological assessments are needed to confirm our results.

Conflict of interest statement

The authors have no conflict of interest to declare.

Funding

This work was in part supported by funds of the "RFO-Ricerca Fondamentale Orientata" of the University of Bologna, Italy

References

[1] Organ Procurement and Transplantation Network. Liver transplantation in the United States, 1999–2008. Available at: http://optn.transplant.hrsa.gov/ar2009/chapter_iv_AR_cd.htm?cp=5.
[2] Adam R, McMaster P, O'Grady JG, et al. European Liver Transplant Association. Evolution of liver transplantation in Europe: report of the European Liver Transplant Registry. Liver Transplantation 2003;9:1231–43.
[3] Wali M, Harrison RF, Gow PJ, et al. Advancing donor liver age and rapid fibrosis progression following transplantation for hepatitis C. Gut 2002;51:248–52.
[4] Mutimer DJ, Gunson B, Chen J, et al. Impact of donor age and year of transplantation on graft and patient survival following liver transplantation for hepatitis C virus. Transplantation 2006;81:7–14.
[5] Rayhill SC, Wu YM, Katz DA, et al. Older donor livers show early severe histological activity, fibrosis, and graft failure after liver transplantation for hepatitis C. Transplantation 2007;84:331–9.
[6] Gane E. The natural history and outcome of liver transplantation in hepatitis C virus-infected recipients. Liver Transplantation 2003;9:S28–34.
[7] Forman LM, Lewis JD, Berlin JA, et al. The association between hepatitis C infection and survival after orthotopic liver transplantation. Gastroenterology 2002;122:889–96.
[8] Burra P, Hepatitis C. Seminars in Liver Disease 2009;29:53–65.
[9] Ercolani G, Grazi GL, Ravaioli M, et al. Histological recurrent hepatitis C after liver transplantation: outcome and role of retransplantation. Liver Transplantation 2006;12:1104–11.
[10] Rowe IA, Barber KM, Birch R, et al. Retransplantation for graft failure in chronic hepatitis C infection: a good use of a scarce resource? World Journal of Gastroenterology 2010;16:5070–6.
[11] Wiesner RH, Sorrell M, Villamil F. International Liver Transplantation Society Expert Panel. Report of the first International Liver Transplantation Society expert panel consensus conference on liver transplantation and hepatitis C. Liver Transplantation 2003;9:S1–9.
[12] Carrión JA, Navasa M, García-Retortillo M, et al. Efficacy of antiviral therapy on hepatitis C recurrence after liver transplantation: a randomized controlled study. Gastroenterology 2007;132:1746–56.
[13] Samuel D, Bizollon T, Feray C, et al. Interferon-alpha 2b plus ribavirin in patients with chronic hepatitis C after liver transplantation: a randomized study. Gastroenterology 2003;124:642–50.
[14] Cescon M, Grazi GL, Cucchetti A, et al. Predictors of sustained virological response after antiviral treatment for hepatitis C recurrence following liver transplantation. Liver Transplantation 2009;15:782–9.
[15] Sharma R, Ryan C, Safadjou S, et al. Response to antiviral therapy in liver transplant recipients with recurrent hepatitis C viral infection: a single center experience. Clinical Transplantation 2010;24:104–11.
[16] Hanouneh IA, Miller C, Aucejo F, et al. Recurrent hepatitis C after liver transplantation: on-treatment prediction of response to peginterferon/ribavirin therapy. Liver Transplantation 2008;14:53–8.
[17] Giostra E, Kullak-Ublick GA, Keller W, et al. Ribavirin/interferon-alpha sequential treatment of recurrent hepatitis C after liver transplantation. Transplant International 2004;17:169–76.
[18] Heydtmann M, Freshwater D, Dudley T, et al. Pegylated interferon alpha-2b for patients with HCV recurrence and graft fibrosis following liver transplantation. American Journal of Transplantation 2006;6:825–33.
[19] Kornberg A, Hommann M, Tannapfel A, et al. Long-term combination of interferon alfa-2b and ribavirin for hepatitis C recurrence in liver transplant patients. American Journal of Transplantation 2001;1:350–5.
[20] Stravitz RT, Shiffman ML, Sanyal AJ, et al. Effects of interferon treatment on liver histology and allograft rejection in patients with recurrent hepatitis C following liver transplantation. Liver Transplantation 2004;10:850–8.
[21] Walter T, Scoazec JY, Guillaud O, et al. Long-term antiviral therapy for recurrent hepatitis C after liver transplantation in nonresponders: biochemical, virological, and histological impact. Liver Transplantation 2009;15:54–63.
[22] Ikegami T, Taketomi A, Soejima Y, et al. The benefits of interferon treatment in patients without sustained viral response after living donor liver transplantation for hepatitis C. Transplantation Proceedings 2009;41:4246–52.
[23] Kamath PS, Wiesner RH, Malinchoc M, et al. A model to predict survival in patients with end-stage liver disease. Hepatology 2001;33:464–70.
[24] Simmonds P, Alberti A, Alter HJ, et al. A proposed system for the nomenclature of hepatitis C viral genotypes. Hepatology 1994;19:1321–4.

[25] Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. Journal of Hepatology 1995;22:696–9.
[26] Lionetti R, Tisone G, Palmieri G, et al. Maintenance ribavirin monotherapy delays fibrosis progression in liver transplant recipients with recurrent hepatitis C at high risk of progression. Digestive and Liver Disease 2010;42:297–303.
[27] Tisone G, Orlando G, Cardillo A, et al. Complete weaning off immunosuppression in HCV liver transplant recipients is feasible and favourably impacts on the progression of disease recurrence. Journal of Hepatology 2006;44:702–9.
[28] Ballarin R, Cucchetti A, Spaggiari M, et al. Long-term follow-up and outcome of liver transplantation from anti-hepatitis C virus-positive donors: a European multicentric case–control study. Transplantation 2011;91:1265–72.
[29] Saab S, Ghobrial RM, Ibrahim AB, et al. Hepatitis C positive grafts may be used in orthotopic liver transplantation: a matched analysis. American Journal of Transplantation 2003;3:1167–72.
[30] Velidedeoglu E, Desai NM, Campos L, et al. The outcome of liver grafts procured from hepatitis C-positive donors. Transplantation 2002;73:582–7.
[31] Bizollon T, Pradat P, Mabrut JY, et al. Benefit of sustained virological response to combination therapy on graft survival of liver transplanted patients with recurrent chronic hepatitis C. American Journal of Transplantation 2005;5:1909–13.
[32] 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 Transplantation 2006;12:1067–76.
[33] Neumann UP, Berg T, Bahra M, et al. Fibrosis progression after liver transplantation in patients with recurrent hepatitis C. Journal of Hepatology 2004;41:830–6.