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Impact of Viral Eradication with Sofosbuvir-based Therapy on the Outcome of Post-Transplant Hepatitis C with Severe Fibrosis

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**Impact of Viral Eradication with Sofosbuvir-based Therapy on the Outcome
of Post-Transplant Hepatitis C with Severe Fibrosis**

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HCV, hepatitis C virus; LT, liver transplantation; Peg-IFN, pegylated-interferon; SVR, sustained virological response; SVR12, sustained virological response at week 12 after the end of treatment; DAAs, direct-acting antiviral agents; LS, liver stiffness; kPa, kilopascal; EOT, end of therapy; *IL28B*, *Interleukin 28B*; HLA, human leukocyte antigen; AE, adverse event; AST, aspartate aminotransferase; APRI, AST to platelet ratio index; ALT, alanine aminotransferase; SVR24, sustained virological response at week 24 after the end of treatment; ROC, receiver operating characteristic; MELD, Model for End-stage Liver Disease; F, fibrosis; ITT, intention to treat.

Conflict of interest

MR is an Advisory Board member for AbbVie, BMS, Gilead, Janssen, MSD.
GMS is an Advisory Board member for AbbVie, Gilead and MSD.
SM is a Regional Advisory Board member for Gilead.
The other Authors who have taken part in this study declared no conflict of interest.

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ABSTRACT

Background & Aims. Several studies have shown that new direct-acting-antivirals (DAAs) maintain their efficacy in liver transplant (LT) recipients with severe HCV recurrence. We determined the clinical impact of Sofosbuvir/Ribavirin in LT through the changes in liver function and fibrosis state at 24 and 48 weeks after treatment.

Methods. Between 06/2014 and 07/2015, 126 patients (30 F3, 96 F4 Metavir stage) were enrolled to receive sofosbuvir+ribavirin (24 weeks, 118 patients) or sofosbuvir+simeprevir+ribavirin (12 weeks, 8 patients); treatment was initiated at a median time of 4.3 years from LT. Median follow-up after therapy completion was 461 days.

Results. All 30 F3 patients achieved a sustained virological response at week 24 after treatment (SVR24), and showed a distinct amelioration of the AST-to-platelet-ratio-index (APRI), FIB-4 and liver stiffness at elastography by week 24 post-therapy, which were maintained at week 48.

Of the 96 F4 cirrhotic patients, 72 (75%) achieved SVR24 accompanied by significant improvement of liver function, which was maintained at week 48 (Child B-C 22% baseline, 11% week 24, 7% week 48); APRI, FIB-4, liver stiffness further improved significantly between week 24 and 48 of follow-up. Among the 77 responders (27 F3, 50 F4) who underwent elastography at baseline and at the end of follow-up, 39 (50.6%; 18 F3, 21 F4) exhibited a regression in fibrosis stage. *Conclusion.* At about one-year from the completion of successful sofosbuvir-based therapy, patients with post-LT HCV and severe fibrosis experienced a long-term liver function improvement accompanied by a regression of fibrosis stage in half of them.

Keywords

Liver transplantation; HCV recurrence; direct-acting antivirals; non-invasive fibrosis tests; survival.

Key point box

- 126 patients with post liver transplant HCV and severe fibrosis (30 F3 and 96 F4 Metavir) received a sofosbuvir-based antiviral therapy. The median follow-up after therapy was 461 days.
- All the 30 F3 patients and 72 of the 96 cirrhotics (75%) achieved a sustained virological response at week 24 after treatment.
- HCV-eradicated patients showed a distinct amelioration in AST-to-platelet-ratio-index, FIB-4 and liver stiffness at week 24 after therapy, which persisted in F3 patients and further improved in F4 ones at week 48.
- In transient elastography, the fibrosis stage regressed in half of the HCV-eradicated patients at week 48 after therapy.

INTRODUCTION

Hepatitis C virus (HCV) is the leading cause of cirrhosis and hepatocellular carcinoma and the most common indication for liver transplantation (LT) in Europe and United States [1].

Infection recurs almost universally in patients who have detectable serum HCV RNA at LT and it is the main cause of graft failure and patient death [2]. Successful HCV eradication is associated with reduced disease progression, and improved liver allograft and recipient survival [3].

Until few years ago, pegylated-interferon (Peg-IFN) with ribavirin was the only treatment for HCV recurrence post-LT; however its use was limited by poor efficacy and beset with significant adverse events [4]. The addition of first generation protease inhibitors, boceprevir and telaprevir, doubled the rates of sustained virological response (SVR) at 12 weeks (SVR12) in HCV genotype 1, but was aggravated by unacceptable toxicity and drug-drug interactions [5]. A major breakthrough has been the advent of second generation direct-acting antiviral agents (DAAs), which interact with different HCV functions and have markedly improved antiviral efficacy and tolerability [6], leading to HCV eradication also in patients with severe recurrent hepatitis C after LT.

The therapeutic potential of the prototype sofosbuvir was quickly perceived; the combinations of sofosbuvir with ribavirin \pm Peg-IFN and of sofosbuvir with simeprevir \pm ribavirin were reported in early international studies to achieve 59% to 88% SVR12 rates [7, 8]. A sofosbuvir compassionate use program was activated in Italy between June 2014 and December 2014; simeprevir became available later, in February 2015.

The primary target of early studies was the virologic efficacy and the tolerability of DAAs in order to meet the urgent demand from transplant patients. In this single-center study, we report the clinical outcome of therapy in a large series of patients with severe fibrosis due to hepatitis C recurrence; the majority were treated with sofosbuvir plus ribavirin, a minority with sofosbuvir plus simeprevir plus ribavirin. We focused on the changes in liver function

parameters and in non-invasive tests of fibrosis up to 48 weeks after the end of successful treatment.

PATIENTS AND METHODS

Study population

Out of 273 surviving HCV recipients with persistent viremia following LT performed in Turin (Italy) since January 2000, between June 2014 and May 2015 we enrolled 126 patients with recurrent hepatitis C who exhibited a Metavir staging score ≥ 3 , and a creatinine clearance ≥ 30 mL/min. The severity of fibrosis was determined within 6 months from treatment by liver biopsy or by transient elastography [liver stiffness (LS) cut-off values of 9.5 kilopascal (kPa) and 12.5 kPa corresponding, respectively, to Metavir F3 and F4 fibrosis stages] [9]. Only transient elastography measurements with a success rate of at least 60% were considered valid.

From June 2014 to December 2014 patients were treated in the context of the sofosbuvir national compassionate use program; each treatment was approved by the local ethic committee (Comitato Etico Interaziendale A.O.U. Città della Salute e della Scienza di Torino—A.O. Ordine Mauriziano—A.S.L. TO1) and the patients provided written informed consent. By the Italian legislation, Regional Transplantation Centers are the lawful custodians of the recipient/donor biomedical data for clinical and for research purposes. All study procedures were compliant with the ethical standards of the 2000 Declaration of Helsinki as well as the Declaration of Istanbul 2008.

The demographic and baseline characteristics of the study population and their donors are shown in Table 1.

Study protocol

The treatment regimens consisted of sofosbuvir 400 mg once daily and ribavirin twice daily (at a dose of 1000 mg daily with body weight <75 kg, or 1200 mg daily with body weight ≥75 kg, adjusted according to haemoglobin level and renal function) for 24 weeks, or sofosbuvir 400 mg once daily, ribavirin twice daily (weight-based) and simeprevir 150 mg once daily for 12 weeks. Epoetin-alfa at 20,000–40,000 units/week was administered subcutaneously to patients with haemoglobin levels <10 g/dL.

Patients underwent clinical and laboratory assessment [HCV RNA by polymerase chain reaction technique with AmpliPrep®/COBAS TaqMan® HCV version 2 (Roche Molecular Systems, Inc., Branchburg, NJ, US), complete blood cell count, liver and kidney function tests, serum electrolytes, serum levels of immunosuppressive drugs] at baseline, weekly for the first month, then monthly while on treatment and at 1, 3, 6 and 12 months after the end of therapy (EOT). No viral resistance monitoring was performed.

At baseline, all patients were tested for HCV genotype (reverse hybridization line probe assay, INNO-LIPA, Innogenetics, Ghent, Belgium), *Interleukin 28B* (*IL28B*) rs12979860 C/T and rs8099917 T/G polymorphisms (Custom TaqMan® Allelic Discrimination Kit, Life Technologies Applied Biosystems®, Thermo Fisher Scientific, Waltham, MA, US) and serum 25-OH vitamin D level (fully automated chemiluminescence immunoassay, ADVIA Centaur® Vitamin D Total assay, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). Human leukocyte antigen (HLA) DRB1 typing was collected as well (performed before transplant, by serology methods until 2006 and then by molecular low-resolution), paying particular attention to HLA-DRB1*11 phenotype [10]. Adverse events (AE), drug dose modifications, use of growth factors, blood transfusions, and need for hospitalisation during treatment were recorded.

To assess the changes in fibrosis evolution in virological responders, transient elastography was performed at 24 and 48 weeks from EOT. The FIB-4 index and the

aspartate aminotransferase (AST) to platelet ratio index (APRI) were calculated at baseline and 24 and 48 weeks after EOT on fasting blood samples. The following formulas were used. $FIB-4 = [age \text{ (years)} \times AST \text{ (IU/L)}] / [platelets \text{ (} 10^9/L) \times alanine \text{ aminotransferase (ALT)}^{1/2} \text{ (IU/L)}]$; a score of <1.45 and >3.25 should identify patients who, respectively, have moderate or significant fibrosis [11]. $APRI = [AST \text{ (IU/L)} / upper \text{ limit of normal (IU/L)}] \times 100 / platelets \text{ (} 10^9/L)$; severe fibrosis was considered unlikely with an $APRI \leq 0.7$ and cirrhosis likely with an $APRI \geq 1$ [12]. Upper limits of normal for our laboratory are: ALT 40 IU/L for male and 35 IU/L for female; AST 45 IU/L for male and 30 IU/L for female. Survival data were collected on May 31, 2016.

Study endpoints

Primary endpoints of the study were survival and clinical outcome of patients who had achieved a sustained virological response at week 24 after EOT (SVR24). To this purpose we considered the variations from baseline to weeks 24 and 48 of: i) liver function parameters; ii) non-invasive tests of fibrosis (APRI, FIB-4, transient elastography). Secondary endpoint was the safety of antiviral therapy.

Statistical analysis

Categorical variables were represented as n (%) and compared using Fisher's exact test. Quantitative variables were shown as median (25th - 75th percentiles) and were compared, as appropriate, by t-test (parametric data), by Mann-Whitney U test (non parametric, unpaired data) or by Wilcoxon signed-rank test (non parametric, paired data). Receiver operating characteristic (ROC) curve analyses were performed to obtain the best possible Model for End-stage Liver Disease (MELD) and Child scores, in order to identify beforehand those patients who achieved SVR24. Data elaboration was performed using

Prism® version 6 (GraphPad Software Inc., La Jolla, CA, US) and IBM SPSS version 21 (New York, NY, US).

RESULTS

Of the 126 patients who entered the study, 118 received sofosbuvir and ribavirin for 24 weeks and 8 were treated with sofosbuvir, simeprevir and ribavirin for 12 weeks. In the former group, 116 completed the full course of therapy; 2 patients died while on treatment (on day 93 and 151), 4 following the EOT (on day 41, 143, 166 and 328). The median follow-up of the surviving patients after EOT was 461 days (range 278-515 days).

All patients except 1 were Caucasian. Forty-seven percent of the grafts came from donors ≥ 65 year old and/or with macrovesicular steatosis $\geq 15\%$ (suboptimal graft) [13], 8% from hepatitis B core antibody positive and 7.1% from HCV antibody positive donors.

Two patients had been treated with a first-generation HCV protease-inhibitor before LT.

The baseline stage of hepatic fibrosis (F) was evaluated in 28 patients by liver biopsy according to Metavir score (3 F3, 25 F4), in 12 patients by combined liver biopsy and transient elastography (12 F4) and in 86 patients by transient elastography only (27 F3 and 59 F4). The median LS was: 11 kPa [interquartile 10.1-12] in the F3 group and 21.8 kPa [17-34.8] in the F4 group. Thirty-four patients had diabetes mellitus, 33 were insulin dependent at the beginning of antiviral therapy.

The 8 patients treated with sofosbuvir, simeprevir and ribavirin were immunosuppressed with tacrolimus. The HCV genotype was 1a in two and 1b in six. Seven patients had fibrosis Metavir F3 and 1 F4 (Child A5, MELD 7), with median HCV RNA 6.56 log₁₀ IU/mL.

Further demographic and baseline characteristics are presented in **Table 1**.

Four weeks after antiviral therapy was started, a statistically significant decrease was observed in the AST and ALT median values from 67 IU/L [45-114] to 24 IU/L [18-33], $p < 0.001$, and 58 IU/L [40-100] to 18 IU/L [13-26], $p < 0.001$, respectively. Transaminase

values normalized (**Figure S1**) and remained normal throughout the follow-up in all eradicated patients but nine, who had persistently elevated enzymes (less than twice upper limit of normal). Six of these latter patients underwent a liver biopsy which showed minimal histological changes without evidence of rejection.

The overall SVR24 rate was 81.0% (102/126 patients, 95% confidence interval 73.2-86.9) by intention to treat (ITT) analysis. The SVR24 rate was significantly higher in F3 compared with F4 patients (30/30, 100% vs 72/96, 75%, $p=0.001$); in patients tolerating $\geq 80\%$ of the full weight-based ribavirin dose ($n=104$) compared with those tolerating $<80\%$ of the dose ($n=22$) (85.6% vs 59.0%, $p=0.01$) and in patients with preserved liver function (MELD ≤ 11 and Child $\leq A6$, according to ROC curves) (**Figure S2**).

Two cirrhotic patients who became HCV RNA negative on therapy, died for multiresistant bacteria-related septic shock; two others died of liver failure after the EOT: one, 68 years old, had achieved a sustained virological response at week 4 after EOT, the other, 44 years old, had achieved SVR12 and was on the list for retransplantation.

Twenty patients experienced a relapse of HCV (17 at week 4, 2 at week 12 and 1 at week 24 of follow-up; the latter cleared HCV RNA at week 4 on therapy and normalized transaminases at week 1). All relapsers were cirrhotic (9 patients Child A, 10 Child B and 1 Child C). They had a median pre-treatment MELD of 13 and had received sofosbuvir and ribavirin; the majority of them were *IL28B* non-C/C genotype (80%) and experienced to previous antiviral therapy (65%) (**Tables S1 and S2**). Basal median LS was 28.0 kPa in the relapsers and 20.4 kPa in the cirrhotics who achieved viral eradication ($p=0.01$). APRI and FIB-4 were significantly higher in relapsers compared with responders at baseline (3.27 vs 1.92 and 8.45 vs 5.95, respectively; $p < 0.01$ for both) (**Table 2**).

From baseline to EOT, the median Child improved in relapsers from B7 to A6 ($p=0.014$), while the median MELD remained 13. Two patients died at 138 and 160 days after relapse due to progressive end-stage liver disease. Seventeen patients started a second treatment

course, at a median of 17 weeks (range 5-43 weeks) from the relapse. No significant variation in liver function tests was observed between relapse and retreatment. One patient refused retreatment.

Overall, in the cirrhotic group, 20 patients (20.8%) had a stable MELD from baseline to the last available follow-up visit and 43 (44.8%) improved the score of at least 1 point; 46 patients (47.9%) had a stable Child and 35 (36.5%) improved the score of at least 1 point.

Clinical outcomes in patients who achieved viral eradication

In the F3 patients, liver and kidney function remained normal (**Table S3**). APRI and FIB-4 were significantly lower at week 24 after EOT, compared with pre-treatment median values (1.07 to 0.41, $p < 0.001$, and 3.26 to 2.08, $p < 0.001$, respectively); these indexes remained stable in the 28 patients who reached 48 weeks of follow-up (**Figure 1, Panels A and C**).

LS (available in 27 patients) improved from 11.0 kPa [10.1-12.0] to 7.7 kPa [6.6-10.2], $p < 0.001$, at week 24 and remained stable at week 48 (7.8 kPa [7.0-10.6], $p = 0.65$ vs week 24) (**Figure 2, Panel A**). In 18 patients (66.7%) LS decreased below the 9.5 kPa cut-off for F3 at week 48.

At week 24 after EOT, the 72 F4 patients who had eradicated HCV, exhibited a significant improvement in platelets count, bilirubin and albumin levels, degree of ascites and Child score at pair wise comparison with baseline values [the rate of Child A was 77.8% (56/72) at baseline and 88.9% (64/72) at week 24 of follow-up]; kidney function and MELD score did not vary. Seventy-one of the 72 responders reached 48 weeks of follow-up and showed a further significant increase in platelets count; albumin, ascites, and Child score [93% (66/71) Child A] remained stable (**Table 3**).

Both APRI and FIB-4 decreased significantly at week 24 after EOT compared with baseline values (1.92 to 0.55, $p < 0.001$, and 5.95 to 3.15, $p < 0.001$, respectively), and further

decreased at week 48, compared with 24 week values (APRI 0.51, $p < 0.001$; FIB-4 3.12, $p < 0.001$) (**Figure 1, Panels B and D**). Fifty-one of the 72 patients underwent transient elastography at baseline and at SVR24; the median LS improved from 20.4 kPa [16.0-33.8] to 17.5 kPa [11.1-25.7], $p < 0.001$ (**Figure 2, Panel B**). Fifty patients underwent transient elastography also at week 48 after EOT; the median LS (14.0 kPa [10.0-21.3]) significantly decreased compared with week 24 ($p = 0.001$); in 21 of them (42%) the LS fell below the 12.5 kPa cut-off.

In 39 (50.6%) of the 77 F3-F4 responders pooled together, the fibrosis stage had regressed in transient elastography by at least one Metavir stage at week 48 after EOT. No patients developed hepatocellular carcinoma in the period of observation.

Thirteen out of the 20 cirrhotic responders with large oesophageal varices at baseline, underwent a second 48 week follow-up endoscopy; no change in the size of varices was observed.

Safety

AE are summarized in **Table S4**.

During therapy, seven cirrhotic patients were hospitalised: two patients for grade 3 encephalopathy, two for severe variceal bleeding and three for sepsis in decompensated liver disease. Six cirrhotic patients died of complications of end-stage liver disease, unrelated to antiviral therapy.

Three patients discontinued ribavirin for less than 1 week due to diffuse macular skin rash and no one discontinued therapy permanently due to AE.

Because of anemia and/or compromised renal function, 34.1% (43/126) of the patients were treated with a non weight-based escalating dose of ribavirin, starting from 400 mg daily and 11.1% (14/126) required dosage reduction; however, 82.5% (104/126) received at least 80% of the weight-based dosage. Median daily ribavirin dose was 1000 mg from

baseline to EOT and median haemoglobin level declined from 12.8 g/dL at baseline to 10.9 g/dL at week 4 and remained stable around 10.8 g/dL from week 8 to week 24 (**Figure S3**). In 60 patients (47.6%) we measured at least one haemoglobin level of less than 10 g/dL and in 12 (9.5%) of less than 8.5 g/dL; 45 patients (35.7%) received epoetin and 14 (11.1%) required blood transfusion.

Minimal dose adjustments in the calcineurin inhibitors were required throughout the treatment in 64% of the patients (27% increased, 12% decreased and 25% both decreased and increased the dose) to maintain stable trough levels. No biopsy-proven acute rejection occurred.

DISCUSSION

The advent of highly effective and well tolerated DAAs against the HCV is revolutionizing the outcome of LT for hepatitis C. In this setting, early treatment after surgery is expected to be the most cost-effective strategy for patients who are still viremic at LT [10, 14], nevertheless DAAs are providing rescue also to residual cohorts of re-infected transplant recipients who developed advanced HCV disease [7, 8, 15]. When sofosbuvir became available in Italy in June 2014, these patients received priority access to the new therapy through a compassionate use program. A majority were treated with sofosbuvir and ribavirin; a small minority received the combination of sofosbuvir, ribavirin and simeprevir, which became available almost at the end of the enrollment period.

After a median follow-up of 461 days from EOT, 102 (81%) of our 126 patients are free of HCV and clinically stable. SVR was obtained also in a high proportion (95%) of the 21 patients with genotype 3, though this genotype is the most difficult to treat in the ordinary clinical context. All treatment failures, excluding deceased patients, were due to virologic relapses and all relapses, except one, occurred within the first 12 weeks after EOT.

Response to therapy was influenced by the stage of fibrosis and by intolerance to ribavirin:

75% of the patients with cirrhosis achieved an SVR compared to 100% of those with F3 fibrosis and among the 22 patients who tolerated less than 80% of the optimal ribavirin dosage, only 59% obtained an SVR. As expected, anemia was the main complication of ribavirin.

Considering the higher proportion of patients with cirrhosis in this study, our results appear better than the 70% rate of SVR12 reported by Charlton [16] in a smaller series of 40 patients recruited in a multicenter study, of whom only 40% were cirrhotics; possibly the management of the patients in a single center adopting a common approach may lead to more uniform results. Though our results are distinctly better than the 43% response reported by Forns et al [7], their series included a mixed population of compensated and decompensated cirrhotics in which a substantial number of patients had a higher MELD score and more severe disease than our patients.

There were in our study six deaths due to complications of the liver disease, two occurring in patients who had cleared the HCV. The deceased patients had a higher MELD score, ranging from 14 to 19, in confirmation that a deteriorated liver function may prevent clinical improvement despite the antiviral efficacy of treatment [17]; vice versa a preserved liver function expressed by a Child Pugh score ≤ 6 and a MELD ≤ 11 was associated with SVR. In our 72 cirrhotic responders, SVR24 was associated with the normalization of aminotransferases in almost all patients, accompanied by a decrease of bilirubin and an increase of albumin levels; renal function deteriorated in none and ascites disappeared in three quarters of cases. The platelet count increased throughout therapy, presumably related to the administration of epoetin and to an increased production of endogenous erythropoietin, secondary to ribavirin-induced anemia [18]. The platelet number diminished to baseline values at week 12 post-therapy, but then climbed back significantly at week 24 and further increased at week 48; this trend and the regression of ascites might be expression of an initial reduction in portal hypertension.

The APRI and FIB-4 indexes significantly improved throughout the follow-up; as aminotransferases and platelets are major components for their calculation, the improvement can be explained by the normalization of the enzymes and by the increase in the platelet count.

LS values were significantly reduced at the 6th month of follow-up; this early improvement presumably resulted for a large extent from the decrease of inflammation and edema which may artificially alter elastography. Interestingly, however, LS values showed a further significant decrease during the subsequent 6 month follow-up in patients with cirrhosis.

Considering that artefacts due to liver inflammation could no longer be implicated, it is plausible that the observed continuing decrease of LS after the SVR24 corresponded to a genuine progressive reduction of fibrosis. In agreement with previous findings of fibrosis reversion in non-transplant patients with HCV cirrhosis who responded to interferon-therapy [19-21], our data would suggest that regression of fibrosis starts early and may become already measureable by transient elastography in the first year after therapy.

In conclusion, our experience in a large single center LT cohort of advanced recurrent HCV disease, confirms the major therapeutic impact of sofosbuvir with ribavirin, extending also to difficult-to-treat genotype 3 patients.

We acknowledge that a new wave of more efficacious DAA combinations is now available to treat hepatitis C [8, 15, 22]. Nevertheless, this study provides a paradigm of the excellent clinical outcome and of the potential for disease reversibility after successful DAA therapy in the transplant setting.

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For Peer Review

Table 1. Demographic and baseline characteristics of recipients and donors

Recipient features (N=126)		
Age (years)		60 [54-66]
Male		99 (78.6%)
Body Mass Index (Kg/m ²)		24.6 [22.5–26.9]
25-OH vitamin D ^Λ (<20 ng/mL)		71 (59.2%)
HLA-DRB1*11 positive phenotype		34 (27.0%)
IL28B genotype		
rs 12979860 C/C		32 (25.4%)
rs 8099917 T/T		59 (46.8%)
Previous antiviral treatment		83 (65.9%)
Antiviral treatment after LT		57 (45.2%)
Immunosuppressive drug		
CsA – Tac – MMF – other		50 (39.7%) – 56 (44.4%) – 56 (44.4%) – 5 (4.0%)
Time since LT (years)		4.3 [2.3–7.2]
HCV genotype		
1a - 1b - 1 (unspecified)		17 (13.5%) – 70 (55.6%) – 6 (4.8%)
2 - 3 – 4		6 (4.8%) – 21 (16.7%) – 6 (4.8%)
Baseline HCV RNA (log ₁₀ IU/mL)		6.40 [6.10–6.66]
≥ 10 ⁶ IU/mL		101 (80.2%)
Corresponding Metavir fibrosis stage		
F3 – F4		30 (23.8%) – 96 (76.2%)
Haemoglobin (g/dL)		12.8 [11.4–14.4]
Platelets count (10 ⁹ /L)		99 [67-140]
Liver enzymes (IU/L)		
Aspartate aminotransferase		60 [40-101]
Alanine aminotransferase		69 [45-116]
γ-Glutamyltransferase		75 [41-152]

Total bilirubin (mg/dL)	1.1 [0.7–1.8]
INR	1.07 [0.98–1.22]
Albumin (g/dL)	3.9 [3.6–4.3]
eGFR* (mL/min)	70 [54-87]
MELD score[§]	10 [8-13]
Child class[§] A – B – C	65 (67.7%) – 27 (28.1%) – 4 (4.2%)
Ascites[§]	38 (39.6%)
Encephalopathy[§]	12 (12.5%)
Presence of varices[§]	26 (27.1%)
Donor features (N=126)	
Age (years)	62 [50–71]
Male	69 (54.8%)
Split liver graft	2 (1.6%)
Graft quality[^] suboptimal[#]	57 (47.5%)
Cold ischemia time (minutes)	486 [424–545]
Donor/Recipient match	
D-MELD	982 [666–1303]

Numerical variables are expressed as median [Q1-Q3]

Categorical variables are expressed as numbers (prevalence, %)

[^]6 missing data

^{*}Chronic Kidney Disease Epidemiology Collaboration equation

[§]In 96 cirrhotic patients

[#]According to Salizzoni et al, Transpl Int 2003

Abbreviations: CsA, cyclosporine; D-MELD, donor age x recipient MELD; eGFR, estimated glomerular filtration rate; F, fibrosis; HCV, hepatitis C virus; HLA, human leukocyte antigen; *IL28B*, *Interleukin 28B*; LT, liver transplantation; MELD, Model for End-stage Liver Disease; MMF, mycophenolate mofetil; Tac, tacrolimus.

Table 2. Liver function tests and non-invasive markers of fibrosis at baseline and at the end of therapy in responder versus relapser cirrhotic patients

BASELINE	Responders (n=72)	Relapsers (n=20)	p[#]
APRI	1.92 [1.00-3.27]	3.27 [1.72-5.51]	0.007
FIB-4	5.95 [3.60-11.10]	8.45 [7.17-16.08]	0.009
Liver stiffness* (kPa)	20.4 [16.0-33.8]	28.0 [21.6–44.4]	0.01
Child score	5 [5-6]	7 [6-8]	0.002
MELD score	10 [8-12]	13 [9-16]	0.02
EOT	Responders (n=72)	Relapsers (n=20)	p[#]
APRI	0.46 [0.29-0.76]	0.83 [0.57-1.22]	0.02
FIB-4	3.25 [1.90-4.30]	4.80 [3.71-7.42]	0.01
Liver stiffness* (kPa)	17.5 [11.1-25.7]	23.4 [18.3-39.0]	0.09
Child score	5 [5-6]	6 [5-7]	0.19
MELD score	11 [9-14]	13 [11-17]	0.05

Numerical variables are expressed as median [Q1-Q3]

*Liver stiffness was available at baseline in 51 responders and 17 relapsers; at EOT in 51 responders and 13 relapsers

[#]p-values calculated according to Wilcoxon test for paired data

Abbreviations: APRI, AST to platelet ratio index; EOT, end of therapy; kPa, kilopascal; MELD, Model for End-stage Liver Disease.

Table 3. Clinical and biochemical variations in long-term responder cirrhotic patients

	Baseline	EOT	SVR12	SVR24	SVR48	p [^]	p [§]
	(N=72)	(N=72)	(N=72)	(N=72)	(N=71°)		
AST (IU/L)	69 [47-97]	23 [20-28]	27 [22-35]	24 [18-32]	21 [18-28]	<0.001	0.13
ALT (IU/L)	59 [41-90]	15 [11-23]	18 [14-27]	16 [12-25]	17 [12-27]	<0.001	0.02
Bilirubin (mg/dL)	1.1 [0.8-1.5]	1.4 [1.1-2.2]	0.9 [0.6-1.2]	0.8 [0.6-1.2]	0.9 [0.6-1.1]	<0.001	0.74
INR	1.06 [0.99-1.20]	1.11 [1.07-1.24]	1.09 [1.02-1.24]	1.08 [1.02-1.22]	1.11 [1.07-1.23]	0.03	0.007
Albumin (g/dL)	3.9 [3.6-4.3]	4.1 [3.8-4.4]	4.1 [3.7-4.3]	4.2 [3.8-4.4]	4.3 [4.0-4.5]	0.02	0.31
Creatinine (mg/dL)	1.1 [0.9-1.3]	1.1 [0.9-1.3]	1.0 [0.9-1.3]	1.1 [1.0-1.3]	1.1 [0.9-1.3]	0.36	0.12
eGFR* (mL/min)	68 [52-84]	64 [52-80]	67 [53-83]	63 [52-78]	62 [52-82]	0.26	0.25
Platelets (10 ⁹ /L)	99 [65-134]	131 [86-185]	99 [64-133]	108 [72-142]	112 [68-144]	0.002	0.01
Ascites	22 (30.6%)	12 (16.7%)	8 (11.1%)	7 (9.7%)	7 (9.9%)	0.003	>0.99
Encephalopathy	5 (6.9%)	4 (5.6%)	3 (4.2%)	3 (4.2%)	2 (2.8%)	0.72	>0.99
MELD score	10 [8-12]	11 [9-14]	9 [8-11]	9 [8-11]	10 [8-11]	0.34	0.92
Child score	5 [5-6]	5 [5-6]	5 [5-6]	5 [5-5]	5 [5-5]	<0.001	0.68

Numerical variables are expressed as median [Q1-Q3]

Categorical variables are expressed as numbers (prevalence,%)

p-values calculated according to Wilcoxon test for paired data for numerical variables and according to Fisher's exact test for categorical variables

°71 out of 72 cured patients reached 48 weeks of follow-up after therapy

*Chronic Kidney Disease Epidemiology Collaboration equation

^Baseline versus SVR24

§SVR24 versus SVR48

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; EOT, end of therapy; MELD, Model for End-stage Liver Disease; SVR12, sustained virological response at week 12 after end of therapy; SVR24, sustained virological response at week 24 after end of therapy; SVR48, sustained virological response at week 48 after end of therapy.

FIGURE LEGENDS

Figure 1. Variations of APRI (Panels A and B) and FIB-4 (Panels C and D) from baseline to weeks 24 and 48 after therapy, in F3 and F4 long-term responders. APRI, AST to platelet ratio index; F, fibrosis according to Metavir; SVR24, sustained virological response at week 24 after therapy; SVR48, sustained virological response at week 48 after therapy.

Figure 2. Variations of liver stiffness from baseline to weeks 24 and 48 after therapy in F3 (Panel A) and F4 (Panel B) long-term responders. F, fibrosis according to Metavir; kPa, kilopascal; SVR24, sustained virological response at week 24 after therapy; SVR48, sustained virological response at week 48 after therapy.

**Impact of Viral Eradication with Sofosbuvir-based Therapy on the Outcome
of Post-Transplant Hepatitis C with Severe Fibrosis**

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List of abbreviations in the order of appearance:

HCV, hepatitis C virus; LT, liver transplantation; Peg-IFN, pegylated-interferon; SVR, sustained virological response; SVR12, sustained virological response at week 12 after the end of treatment; DAAs, direct-acting antiviral agents; LS, liver stiffness; kPa, kilopascal; EOT, end of therapy; *IL28B*, *Interleukin 28B*; HLA, human leukocyte antigen; AE, adverse event; AST, aspartate aminotransferase; APRI, AST to platelet ratio index; ALT, alanine aminotransferase; SVR24, sustained virological response at week 24 after the end of treatment; ROC, receiver operating characteristic; MELD, Model for End-stage Liver Disease; F, fibrosis; ITT, intention to treat. ~~AUC, area under the curve.~~

Conflict of interest

MR is an Advisory Board member for AbbVie, BMS, Gilead, Janssen, MSD.

GMS is an Advisory Board member for AbbVie, Gilead and MSD.

SM is a Regional Advisory Board member for Gilead.

The other Authors who have taken part in this study declared no conflict of interest.

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ABSTRACT

Background & Aims. Several studies report have shown that the new direct-acting-antivirals (DAAs) induce maintain viral their efficacy eradication also in liver transplant (LT) recipients with severe HCV recurrence. We determined The the clinical impact of Sofosbuvir/Ribavirin in LT therapy through the changes in on liver function and on fibrosis progression state at 24 and 48 weeks after treatment was evaluated.

Methods. Between 06/2014 and 07/2015, 126 patients (30 F3, 96 F4 Metavir stage) were enrolled to receive sofosbuvir+ribavirin (24 weeks, 118 patients) or sofosbuvir+simeprevir+ribavirin (12 weeks, 8 patients); treatment was initiated at a median time of 4.3 years from LT. Median follow-up after therapy completion was 461 days.

Results. All 30 F3 patients achieved a sustained virological response at week 24 after treatment (SVR24), and showed a distinct amelioration of the AST-to-platelet-ratio-index (APRI), FIB-4 and liver stiffness at elastography by week 24 post-therapy, which were maintained at week 48.

Of the 96 F4 cirrhotic patients, 72 (75%) achieved SVR24 accompanied by a significant improvement of liver function, which was maintained at week 48 (Child B-C 22% baseline, 11% week 24, 7% week 48); APRI, FIB-4, and liver stiffness further improved significantly between week 24 and 48 of follow-up. Among the 77 responders (27 F3, 50 F4) who underwent elastography at baseline and at the end of follow-up, 39 (50.6%; 18 F3, 21 F4) exhibited a regression in fibrosis stage. *Conclusion.* At about one-year from the completion of successful sofosbuvir-based therapy, patients with post-LT HCV and severe fibrosis experienced a long-term liver function improvement accompanied by a regression of fibrosis stage in half of them.

Keywords

Liver transplantation; HCV recurrence; direct-acting antivirals; non-invasive fibrosis tests; survival.

Key point box

- 126 patients with post liver transplant HCV and severe fibrosis (30 F3 **and** 96 F4 Metavir) received a sofosbuvir-based antiviral therapy. **The M**edian follow-up after therapy was 461 days.
- All **the** 30 F3 patients and ~~72/96~~ **72 of the 96** (75%) cirrhotics **(75%)** ~~are currently alive and~~ achieved a sustained virological response at week 24 after treatment.
- HCV-**eradicated** ~~cured~~ patients showed a distinct amelioration in AST-to-platelet-ratio-index, FIB-4 and liver stiffness at week 24 after therapy, which persisted in F3 patients and further improved in F4 ones at week 48.
- **In transient elastography, the fibrosis stage regressed in half of the HCV-eradicated**
~~Overall, a fibrosis stage regression evaluated by transient elastography, occurred in half of the patients at week 48 after therapy.~~

INTRODUCTION

Hepatitis C virus (HCV) is the leading cause of cirrhosis and hepatocellular carcinoma and the most common indication for liver transplantation (LT) in Europe and United States [1]. Infection recurs almost universally in patients who have detectable serum HCV RNA at LT and it is the main cause of graft failure and patient death [2]. Successful HCV eradication is associated with reduced disease progression, and improved liver allograft and recipient survival [3].

Until few years ago, pegylated-interferon (Peg-IFN) with ribavirin was the only treatment for HCV recurrence post-LT; however its use was limited by poor efficacy and beset with significant adverse events [4]. The addition of first generation protease inhibitors, boceprevir and telaprevir, doubled the rates of sustained virological response (SVR) at 12 weeks (SVR12) in HCV genotype 1, but was aggravated by unacceptable toxicity and drug-drug interactions [5]. A major breakthrough has been the advent of second generation direct-acting antiviral agents (DAAs), which interact with different HCV functions and have markedly improved antiviral efficacy and tolerability [6], leading to HCV eradication also in patients with severe recurrent hepatitis C after LT.

The therapeutic potential of the prototype sofosbuvir was quickly perceived; the combinations of sofosbuvir with ribavirin ± Peg-IFN and of sofosbuvir with simeprevir ± ribavirin were reported in early international studies to achieve 59% to 88% SVR12 rates [7, 8]. A sofosbuvir compassionate use program was activated in Italy between June 2014 and December 2014; simeprevir became available later, in February 2015.

The primary target of early studies was the virologic efficacy and the tolerability of DAAs in order to meet the urgent demand from transplant patients. In this single-center study, we report the clinical outcome of therapy in a large series of patients with severe fibrosis due to hepatitis C recurrence; the majority were treated with sofosbuvir plus ribavirin, a minority with sofosbuvir plus simeprevir plus ribavirin. We focused on the changes in liver function

parameters and in non-invasive tests of fibrosis up to 48 weeks after the end of successful treatment. These studies had short follow-ups and did not deal with the long-term clinical outcome of LT patients who had responded to therapy.

In this single-center study, we collected a large series of patients with post-transplant severe fibrosis due to hepatitis C recurrence; the majority were treated with sofosbuvir plus ribavirin, a minority with sofosbuvir plus simeprevir plus ribavirin. We report the virologic results and focus on the changes observed in liver function parameters and in non-invasive tests of fibrosis up to 48 weeks after the end of successful therapy.

PATIENTS AND METHODS

Study population

Out of 273 surviving HCV recipients with persistent viremia following LT performed in Turin (Italy) since January 2000, between June 2014 and May 2015 we enrolled 126 patients with recurrent hepatitis C who exhibited a Metavir staging score ≥ 3 , and a creatinine clearance ≥ 30 mL/min. The severity of fibrosis was determined within 6 months from treatment by liver biopsy or by transient elastography [liver stiffness (LS) cut-off values of 9.5 kilopascal (kPa) and 12.5 kPa corresponding, respectively, to Metavir F3 and F4 fibrosis stages] [9]. Only transient elastography measurements with a success rate of at least 60% were considered valid.

From June 2014 to December 2014 patients were treated in the context of the sofosbuvir national compassionate use program; each treatment was approved by the local ethic committee (Comitato Etico Interaziendale A.O.U. Città della Salute e della Scienza di Torino—A.O. Ordine Mauriziano—A.S.L. TO1) and the patients provided written informed consent. By the Italian legislation, Regional Transplantation Centers are the lawful custodians of the recipient/donor biomedical data for clinical and for research purposes. All study procedures were compliant with the ethical standards of the 2000 Declaration of

Helsinki as well as the Declaration of Istanbul 2008.

The demographic and baseline characteristics of the study population and their donors are shown in Table 1.

Study protocol

The treatment regimens consisted of sofosbuvir 400 mg once daily and ribavirin twice daily (at a dose of 1000 mg daily with body weight <75 kg, or 1200 mg daily with body weight ≥75 kg, adjusted according to haemoglobin level and renal function) for 24 weeks, or sofosbuvir 400 mg once daily, ribavirin twice daily (weight-based) and simeprevir 150 mg once daily for 12 weeks. Epoetin-alfa at 20,000-40,000 units/week was administered subcutaneously to patients with haemoglobin levels <10 g/dL.

Patients underwent clinical and laboratory assessment [HCV RNA by polymerase chain reaction technique with AmpliPrep®/COBAS TaqMan® HCV version 2 (Roche Molecular Systems, Inc., Branchburg, NJ, US), complete blood cell count, liver and kidney function tests, serum electrolytes, serum levels of immunosuppressive drugs] at baseline, weekly for the first month, then monthly while on treatment and at 1, 3, 6 and 12 months after the end of therapy (EOT). No viral resistance monitoring was performed.

At baseline, all patients were tested for HCV genotype (reverse hybridization line probe assay, INNO-LIPA, Innogenetics, Ghent, Belgium), *Interleukin 28B (IL28B)* rs12979860 C/T and rs8099917 T/G polymorphisms (Custom TaqMan® Allelic Discrimination Kit, Life Technologies Applied Biosystems®, Thermo Fisher Scientific, Waltham, MA, US) and serum 25-OH vitamin D level (fully automated chemiluminescence immunoassay, ADVIA Centaur® Vitamin D Total assay, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). Human leukocyte antigen (HLA) DRB1 typing was collected as well (performed before transplant, by serology methods until 2006 and then by molecular low-resolution), paying particular attention to HLA-DRB1*11 phenotype [10]. Adverse events (AE), drug

dose modifications, use of growth factors, blood transfusions, and need for hospitalisation during treatment were recorded.

To assess the changes in fibrosis evolution in virological responders, transient elastography was performed at 24 and 48 weeks from EOT. The FIB-4 index and the aspartate aminotransferase (AST) to platelet ratio index (APRI) were calculated at baseline and 24 and 48 weeks after EOT on fasting blood samples. The following formulas were used. $FIB-4 = [age \text{ (years)} \times AST \text{ (IU/L)}] / [platelets \text{ (} 10^9/L) \times alanine \text{ aminotransferase (ALT)}^{1/2} \text{ (IU/L)}]$; a score of <1.45 and >3.25 should identify patients who, respectively, have moderate or significant fibrosis [11]. $APRI = [AST \text{ (IU/L)} / upper \text{ limit of normal (IU/L)}] \times 100 / platelets \text{ (} 10^9/L)$; severe fibrosis was considered unlikely with an $APRI \leq 0.7$ and cirrhosis likely with an $APRI \geq 1$ [12]. Upper limits of normal for our laboratory are: ALT 40 IU/L for male and 35 IU/L for female; AST 45 IU/L for male and 30 IU/L for female.

Survival data were collected on May 31, 2016.

Study endpoints

Primary endpoints of the study were survival and clinical outcome of patients who had achieved a sustained virological response at week 24 after EOT (SVR24). To this purpose we considered the variations from baseline to weeks 24 and 48 of: i) liver function parameters; ii) non-invasive tests of fibrosis (APRI, FIB-4, transient elastography).

Secondary endpoint was the safety of antiviral therapy.

Statistical analysis

Categorical variables were represented as n (%) and compared using Fisher's exact test. Quantitative variables were shown as median (25th - 75th percentiles) and were compared, as appropriate, by t-test (parametric data), by Mann-Whitney U test (non parametric, unpaired data) or by Wilcoxon signed-rank test (non parametric, paired data). Receiver

operating characteristic (ROC) curve analyses were performed to obtain the best possible Model for End-stage Liver Disease (MELD) **and** Child scores., ~~and non-invasive fibrosis tests thresholds~~, in order to identify beforehand those patients who achieved SVR24. Data elaboration was performed using Prism® version 6 (GraphPad Software Inc., La Jolla, CA, US) and IBM SPSS version 21 (New York, NY, US).

RESULTS

Of the 126 patients who entered the study, 118 received sofosbuvir and ribavirin for 24 weeks and 8 were treated with sofosbuvir, simeprevir and ribavirin for 12 weeks. In the former group, 116 completed the full course of therapy; 2 patients died while on treatment (on day 93 and 151), 4 following the EOT (on day 41, 143, 166 and 328). The median follow-up of the surviving patients after EOT was 461 days (range 278-515 days).

All patients except 1 were Caucasian. Forty-seven percent of the grafts came from donors ≥65 year old and/or with macrovesicular steatosis ≥15% (suboptimal graft) [13], 8% from hepatitis B core antibody positive donors and 7.1% from HCV antibody positive **donors**.

Two patients had been treated with a first-generation HCV protease-inhibitor before LT. The baseline stage of hepatic fibrosis (F) was evaluated in 28 patients by liver biopsy according to Metavir score (3 F3, 25 F4), in 12 patients by combined liver biopsy and transient elastography (12 F4) and in 86 patients by transient elastography only (27 F3 and 59 F4). The median LS was: 11 kPa [interquartile 10.1-12] in the F3 group and 21.8 kPa [17-34.8] in the F4 group. Thirty-four patients had diabetes mellitus, 33 were insulin dependent at the beginning of antiviral therapy.

~~All~~ **The** 8 patients treated with sofosbuvir, simeprevir and ribavirin were immunosuppressed with tacrolimus. The HCV genotype was 1a in two and 1b in six. Seven patients had fibrosis Metavir F3 and 1 F4 (Child A5, MELD 7), with median HCV RNA 6.56 log₁₀ IU/mL.

Further demographic and baseline characteristics are presented in **Table 1**.

Four weeks after antiviral therapy was started, a statistically significant decrease was observed in the AST and ALT median values from 67 IU/L [45-114] to 24 IU/L [18-33], $p < 0.001$, and 58 IU/L [40-100] to 18 IU/L [13-26], $p < 0.001$, respectively. Transaminase values normalized (**Figure S1**) and remained normal throughout the follow-up in all eradicated patients but nine, whose had persistently elevated enzymes transaminases persisted slightly elevated (less than twice upper limit of normal). Six of these latter patients underwent a liver biopsy which showed minimal histological changes without evidence of rejection. After 1 week of therapy, the median decrease in HCV RNA was $3.87 \log_{10}$ IU/mL in F3 and $3.55 \log_{10}$ IU/mL in F4 group ($p = 0.002$) (**Figures S2 and S3**). The overall SVR24 rate was 81.0% (102/126 patients, 95% confidence interval 73.2-86.9) by intention to treat (ITT) analysis (**Figure 1**). The SVR24 rate was significantly higher in F3 patients compared with F4 patients ones (30/30, 100% vs 72/96, 75%, $p = 0.001$); and in patients tolerating $\geq 80\%$ of the full weight-based ribavirin dose ($n = 104$) compared with those tolerating $< 80\%$ of the dose ($n = 22$) (85.6% vs 59.0%, $p = 0.01$) and in patients with preserved liver function (MELD ≤ 11 and Child $\leq A6$, according to ROC curves) (Figure S2). Two cirrhotic patients who became HCV RNA negative on therapy, died for multiresistant bacteria-related septic shock, two others died of liver failure after the EOT: one, 68 years old, had achieved a sustained virological response at week 4 after EOT, the other, 44 years old, had achieved SVR12 and was on the list for retransplantation. Twenty patients experienced a relapsed of HCV (17 at week 4, 2 at week 12 and 1 at week 24 of follow-up; the latter cleared HCV RNA at week 4 on therapy and normalized transaminases at week 1). All relapsers were cirrhotic (9 patients Child A, 10 Child B and 1 Child C). They had a median pre-treatment MELD of 13 and had received sofosbuvir and ribavirin; a the majority of them were *IL28B* non-C/C genotype (80%) and experienced to previous antiviral therapy (65%) (**Tables S1 and S2**). Basal median LS was 28.0 kPa in

the relapsers and 20.4 kPa in the cirrhotics who achieved viral eradication ($p=0.01$). APRI and FIB-4 were significantly higher in relapsers compared with responders at baseline (3.27 vs 1.92 and 8.45 vs 5.95, respectively; $p < 0.01$ for both) (Table 2).

From baseline to EOT, their median Child improved in relapsers from B7 to A6 ($p=0.014$), while the median MELD remained 13. Two patients died at 138 and 160 days after relapse due to progressive end-stage liver disease. Seventeen patients started a second treatment course, at a median of 17 weeks (range 5-43 weeks) from the relapse. No significant variation in liver function tests was observed between relapse and retreatment. (Table S2). One patient refused retreatment.

Two cirrhotic patients who were HCV RNA negative on therapy, died for multiresistant bacteria-related septic shock; 2 others died of liver failure after the EOT: one, 68 years old, had achieved a sustained virological response at week 4 after EOT, and one, 44 years old, had achieved SVR12 and was on the list for retransplantation.

In the cirrhotic patients, significant predictors of SVR24 at univariate analysis (Figure 2) were MELD ≤ 11 , Child $\leq A6$ and LS ≤ 26 kPa. The cut-offs were chosen according to ROC curves: area under the curve (AUC) for MELD 0.723, $p=0.001$; Child 0.759, $p < 0.001$ and LS 0.751, $p=0.002$ (Figures S4 and S5).

Basal median LS was 28.0 kPa in the relapsers and 20.4 kPa in the cirrhotics who achieved viral eradication ($p=0.01$). APRI and FIB-4 at baseline were significantly higher in relapsers compared with responders (3.27 vs 1.92 and 8.45 vs 5.95, respectively; $p < 0.01$ for both) (Table S3).

Overall, in the cirrhotic group, between baseline and the last available follow-up, 20 patients (20.8%) showed had a stable MELD from baseline to the last available follow-up visit and 43 (44.8%) improved the score of at least 1 point; 46 patients (47.9%) showed had a stable Child and 35 (36.5%) improved the score of at least 1 point.

Clinical outcomes in patients who achieved viral eradication

In the F3 patients, liver and kidney function remained normal (**Table S34**). APRI and FIB-4 were significantly lower at week 24 after EOT, compared with pre-treatment median values (1.07 to 0.41, $p < 0.001$, and 3.26 to 2.08, $p < 0.001$, respectively); these indexes remained stable in the 28 patients who reached 48 weeks of follow-up (**Figure 13, Panels A and C**). LS (available in 27 patients) improved from 11.0 kPa [10.1-12.0] to 7.7 kPa [6.6-10.2], $p < 0.001$, at week 24 and remained stable at week 48 (7.8 kPa [7.0-10.6], $p = 0.65$ vs week 24) (**Figure 24, Panel A**). In 18 patients (66.7%) LS decreased below the 9.5 kPa cut-off for F3 at week 48.

At week 24 after EOT, the 72 F4 patients who ~~had achieved viral eradication~~ **eradicated HCV**, ~~showed~~ **exhibited** a significant improvement in platelets count, bilirubin and albumin levels, degree of ascites and Child score at pair wise comparison with baseline values [the rate of Child A was 77.8% (56/72) at baseline and 88.9% (64/72) at week 24 of follow-up]; kidney function and MELD score did not vary. Seventy-one of the 72 responders reached 48 weeks of follow-up and showed a further significant increase in platelets count; albumin, ascites, and Child score [93% (66/71) Child A] remained stable (**Table 32**).

Both APRI and FIB-4 decreased significantly at week 24 after EOT compared with baseline values (1.92 to 0.55, $p < 0.001$, and 5.95 to 3.15, $p < 0.001$, respectively), and further decreased at week 48, compared with 24 week values (APRI 0.51, $p < 0.001$; FIB-4 3.12, $p < 0.001$) (**Figure 13, Panels B and D**). Fifty-one of the 72 patients underwent transient elastography at baseline and at SVR24; the median LS improved from 20.4 kPa [16.0-33.8] to 17.5 kPa [11.1-25.7], $p < 0.001$ (**Figure 24, Panel B**). Fifty patients underwent transient elastography also at week 48 after EOT; the median LS (14.0 kPa [10.0-21.3]) significantly decreased compared with week 24 ($p = 0.001$); in 21 of them (42%) the LS fell below the 12.5 kPa cut-off.

As evaluated by transient elastography in F3-F4 patients pooled together, **In** 39 (50.6%) of the 77 (50.6%) **F3-F4** responders **pooled together**, the fibrosis stage had regressed in transient elastography by at least one Metavir stage at week 48 after EOT.

No patients developed hepatocellular carcinoma in the period of observation.

Thirteen out of the 20 cirrhotic responders with large oesophageal varices at baseline, underwent a second 48 week follow-up endoscopy; **no change in the size of varices was observed**. ~~which showed no changes.~~

Safety

AE are summarized in **Table S45**.

During therapy, seven cirrhotic patients were hospitalised: two patients for grade 3 encephalopathy, two for severe variceal bleeding and three for sepsis in decompensated liver disease. Six cirrhotic patients died of complications of end-stage liver disease, unrelated to antiviral therapy.

Three patients discontinued ribavirin for less than 1 week due to diffuse macular skin rash and no one discontinued therapy permanently due to AE.

Because of anemia and/or compromised renal function, 34.1% (43/126) of the patients were treated with a non weight-based escalating dose of ribavirin, starting from 400 mg daily and 11.1% (14/126) required dosage reduction; however, 82.5% (104/126) received at least 80% of the weight-based dosage. Median daily ribavirin dose was 1000 mg from baseline to EOT and median haemoglobin level declined from 12.8 g/dL at baseline to 10.9 g/dL at week 4 and remained stable around 10.8 g/dL from week 8 to week 24 (**Figure S36**). In 60 patients (47.6%) we measured at least one haemoglobin level of less than 10 g/dL and in 12 (9.5%) of less than 8.5 g/dL; 45 patients (35.7%) received epoetin and 14 (11.1%) required blood transfusion.

Minimal dose adjustments in the calcineurin inhibitors were required throughout the treatment in 64% of the patients (27% increased, 12% decreased and 25% both decreased and increased the dose) to maintain stable trough levels. No biopsy-proven acute rejection occurred.

DISCUSSION

The advent of highly effective and well tolerated DAAs against the HCV is revolutionizing the outcome of LT for hepatitis C. In this setting, early treatment after surgery is expected to be the most cost-effective strategy for patients who are still viremic at LT [10, 14], nevertheless DAAs are providing rescue also to residual cohorts of re-infected transplant recipients who developed advanced HCV disease [7, 8, 15]. When sofosbuvir became available in Italy in June 2014, these patients received priority access to the new therapy through a compassionate use program. A majority were treated with sofosbuvir and ribavirin; a small minority received the combination of sofosbuvir, ribavirin and simeprevir, which became available almost at the end of the enrollment period.

After a median follow-up of 461 days from EOT, 102 (81%) of our 126 patients are free of HCV and clinically stable. SVR was obtained also in a high proportion (95%) of the 21 patients with genotype 3, though this genotype is the most difficult to treat in the ordinary clinical context. All treatment failures, excluding deceased patients, were due to virologic relapses and all relapses, except one, occurred within the first 12 weeks after EOT.

Response to therapy was independent from gender, HCV genotype, basal viremia, rapid virologic response, HLA DRB1*11 phenotype and *IL28B* polymorphisms, while it was influenced by the stage of fibrosis and by intolerance to ribavirin: 75% of the patients with cirrhosis achieved an SVR compared to 100% of those with F3 fibrosis and among the 22 patients who tolerated less than 80% of the optimal ribavirin dosage, only 59% obtained an SVR. As expected, anemia was the main complication of ribavirin.

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3 Considering the higher proportion of patients with cirrhosis in this study, our results appear
4 better than the 70% rate of SVR12 reported by Charlton [16] in a smaller series of 40
5 patients recruited in a multicenter study, of whom only 40% were cirrhotics; possibly the
6 management of the patients in a single center adopting a common approach may lead to
7 more uniform results. Though our results are distinctly better than the 43% response
8 reported by Forns et al [7], their series included a mixed population of compensated and
9 decompensated cirrhotics in which a substantial number of patients had a higher MELD
10 score and more severe disease than our patients.
11

12 There were in our study six deaths due to complications of the liver disease, two occurring
13 in patients who had cleared the HCV. The deceased patients had a higher MELD score,
14 ranging from 14 to 19, in confirmation that a deteriorated liver function may prevent clinical
15 improvement despite the antiviral efficacy of treatment [17]; vice versa a preserved liver
16 function expressed by a Child Pugh score ≤ 6 and a MELD ≤ 11 was associated with SVR.
17 Unfortunately, the threshold that may predict the lack of a clinical advantage despite the
18 efficacy of therapy is difficult to establish [17].
19

20 In our 72 cirrhotic responders, SVR24 was associated with the normalization of
21 aminotransferases in almost all patients, accompanied by a decrease of bilirubin and an
22 increase of albumin levels; renal function deteriorated in none and ascites disappeared in
23 three quarters of cases. The platelet count increased throughout therapy, presumably
24 related to the administration of epoetin and to an increased production of endogenous
25 erythropoietin, secondary to ribavirin-induced anemia [18]. The platelet number diminished
26 to baseline values at week 12 post-therapy, but then climbed back significantly at week 24
27 and further increased at week 48; this trend and the regression of ascites may **might** be
28 expression of an initial reduction in portal hypertension.
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30 The APRI and FIB-4 indexes significantly improved throughout the follow-up; as
31 aminotransferases and platelets are major components for their calculation, the
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improvement can be explained by the normalization of the enzymes and by the increase in the platelet count.

Transient elastography LS values were significantly reduced at the 6th month of follow-up; this early improvement presumably resulted for a large extent by ~~from~~ the decrease of inflammation and edema which may artificially alter elastography. Interestingly, however, LS values showed a further significant decrease during the subsequent 6 month follow-up in patients with cirrhosis. Considering that artefacts due to liver inflammation could no longer be implicated, it is plausible that the observed continuing decrease of LS after the SVR24 corresponded to a genuine progressive reduction of fibrosis. In agreement with previous findings of fibrosis reversion in non-transplant patients with HCV cirrhosis who responded to interferon-therapy [19-21], our data would suggest that regression of fibrosis starts early and may become already measureable by transient elastography in the first year after therapy.

In conclusion, our experience in a large single center LT cohort of advanced recurrent HCV disease, confirms the major therapeutic impact of sofosbuvir with ribavirin, extending also to difficult-to-treat genotype 3 patients.

We acknowledge that a new wave of more efficacious DAA combinations is now available to treat hepatitis C [8, 15, 22]. Nevertheless, this study provides a paradigm of the excellent clinical outcome ~~and of the potential for disease reversibility after~~ that can be expected with successful DAA therapy in the transplant setting.

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Table 1. Demographic and baseline characteristics of recipients and donors

Recipient features (N=126)		
Age (years)		60 [54-66]
Male		99 (78.6%)
Body Mass Index (Kg/m ²)		24.6 [22.5–26.9]
25-OH vitamin D [⋆] (<20 ng/mL)		71 (59.2%)
HLA-DRB1*11 positive phenotype		34 (27.0%)
IL28B genotype		
rs 12979860 C/C		32 (25.4%)
rs 8099917 T/T		59 (46.8%)
Previous antiviral treatment		83 (65.9%)
Antiviral treatment after LT		57 (45.2%)
Immunosuppressive drug		
CsA – Tac – MMF – other		50 (39.7%) – 56 (44.4%) – 56 (44.4%) – 5 (4.0%)
Time since LT (years)		4.3 [2.3–7.2]
HCV genotype		
1a - 1b - 1 (unspecified)		17 (13.5%) – 70 (55.6%) – 6 (4.8%)
2 - 3 – 4		6 (4.8%) – 21 (16.7%) – 6 (4.8%)
Baseline HCV RNA (log ₁₀ IU/mL)		6.40 [6.10–6.66]
≥ 10 ⁶ IU/mL		101 (80.2%)
Corresponding Metavir fibrosis stage		
F3 – F4		30 (23.8%) – 96 (76.2%)
Haemoglobin (g/dL)		12.8 [11.4–14.4]
Platelets count (10 ⁹ /L)		99 [67-140]
Liver enzymes (IU/L)		
Aspartate aminotransferase		60 [40-101]
Alanine aminotransferase		69 [45-116]
γ-Glutamyltransferase		75 [41-152]

Total bilirubin (mg/dL)	1.1 [0.7–1.8]
INR	1.07 [0.98–1.22]
Albumin (g/dL)	3.9 [3.6–4.3]
eGFR* (mL/min)	70 [54-87]
MELD score[§]	10 [8-13]
Child class[§] A – B – C	65 (67.7%) – 27 (28.1%) – 4 (4.2%)
Ascites[§]	38 (39.6%)
Encephalopathy[§]	12 (12.5%)
Presence of varices[§]	26 (27.1%)
Donor features (N=126)	
Age (years)	62 [50–71]
Male	69 (54.8%)
Split liver graft	2 (1.6%)
Graft quality[^] suboptimal[#]	57 (47.5%)
Cold ischemia time (minutes)	486 [424–545]
Donor/Recipient match	
D-MELD	982 [666–1303]

Numerical variables are expressed as median [Q1-Q3]. Categorical variables are expressed as numbers (prevalence, %).

[^]6 missing data

^{*}Chronic Kidney Disease Epidemiology Collaboration equation

[§]In 96 cirrhotic patients

[#]According to Salizzoni et al, Transpl Int 2003

Abbreviations: CsA, cyclosporine; D-MELD, donor age x recipient MELD; eGFR, estimated glomerular filtration rate; F, fibrosis; HCV, hepatitis C virus; HLA, human leukocyte antigen; IL28B, Interleukin 28B; LT, liver transplantation; MELD, Model for End-stage Liver Disease; MMF, mycophenolate mofetil; Tac, tacrolimus.

Table 2. Liver function tests and non-invasive markers of fibrosis at baseline and at the end of therapy in responder versus relapser cirrhotic patients

BASELINE	Responders (n=72)	Relapsers (n=20)	p[#]
APRI	1.92 [1.00-3.27]	3.27 [1.72-5.51]	0.007
FIB-4	5.95 [3.60-11.10]	8.45 [7.17-16.08]	0.009
Liver stiffness* (kPa)	20.4 [16.0-33.8]	28.0 [21.6-44.4]	0.01
Child score	5 [5-6]	7 [6-8]	0.002
MELD score	10 [8-12]	13 [9-16]	0.02
EOT	Responders (n=72)	Relapsers (n=20)	p[#]
APRI	0.46 [0.29-0.76]	0.83 [0.57-1.22]	0.02
FIB-4	3.25 [1.90-4.30]	4.80 [3.71-7.42]	0.01
Liver stiffness* (kPa)	17.5 [11.1-25.7]	23.4 [18.3-39.0]	0.09
Child score	5 [5-6]	6 [5-7]	0.19
MELD score	11 [9-14]	13 [11-17]	0.05
Numerical variables are expressed as median [Q1-Q3]			
*Liver stiffness was available at baseline in 51 responders and 17 relapsers; at EOT in 51 responders and 13 relapsers			
[#] p-values calculated according to Wilcoxon test for paired data			
Abbreviations: APRI, AST to platelet ratio index; EOT, end of therapy; kPa, kilopascal; MELD, Model for End-stage Liver Disease.			

Table 32. Clinical and biochemical variations in cirrhotic long-term responder cirrhotic patients who achieved viral eradication

	Baseline (N=72)	EOT (N=72)	SVR12 (N=72)	SVR24 (N=72)	SVR48 (N=71°)	p^	p§
AST (IU/L)	69 [47-97]	23 [20-28]	27 [22-35]	24 [18-32]	21 [18-28]	<0.001	0.13
ALT (IU/L)	59 [41-90]	15 [11-23]	18 [14-27]	16 [12-25]	17 [12-27]	<0.001	0.02
Bilirubin (mg/dL)	1.1 [0.8-1.5]	1.4 [1.1-2.2]	0.9 [0.6-1.2]	0.8 [0.6-1.2]	0.9 [0.6-1.1]	<0.001	0.74
INR	1.06 [0.99-1.20]	1.11 [1.07-1.24]	1.09 [1.02-1.24]	1.08 [1.02-1.22]	1.11 [1.07-1.23]	0.03	0.007
Albumin (g/dL)	3.9 [3.6-4.3]	4.1 [3.8-4.4]	4.1 [3.7-4.3]	4.2 [3.8-4.4]	4.3 [4.0-4.5]	0.02	0.31
Creatinine (mg/dL)	1.1 [0.9-1.3]	1.1 [0.9-1.3]	1.0 [0.9-1.3]	1.1 [1.0-1.3]	1.1 [0.9-1.3]	0.36	0.12
eGFR* (mL/min)	68 [52-84]	64 [52-80]	67 [53-83]	63 [52-78]	62 [52-82]	0.26	0.25
Platelets (10⁹/L)	99 [65-134]	131 [86-185]	99 [64-133]	108 [72-142]	112 [68-144]	0.002	0.01
Ascites	22 (30.6%)	12 (16.7%)	8 (11.1%)	7 (9.7%)	7 (9.9%)	0.003	>0.99
Encephalopathy	5 (6.9%)	4 (5.6%)	3 (4.2%)	3 (4.2%)	2 (2.8%)	0.72	>0.99
MELD score	10 [8-12]	11 [9-14]	9 [8-11]	9 [8-11]	10 [8-11]	0.34	0.92
Child score	5 [5-6]	5 [5-6]	5 [5-6]	5 [5-5]	5 [5-5]	<0.001	0.68

Numerical variables are expressed as median [Q1-Q3]. Categorical variables are expressed as numbers (prevalence,%).

p-values calculated according to Wilcoxon test for paired data for numerical variables and according to Fisher's exact test for categorical variables.

°71 out of 72 cured patients reached 48 weeks of follow-up after therapy

*Chronic Kidney Disease Epidemiology Collaboration equation

^Baseline versus SVR24

§SVR24 versus SVR48

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; EOT, end of therapy; MELD, Model for End-stage Liver Disease; SVR12, sustained virological response at week 12 after end of therapy; SVR24, sustained virological response at week 24 after end of therapy; SVR48, sustained virological response at week 48 after end of therapy.

FIGURE LEGENDS

Figure 1. Sustained virological response rates by intention to treat analysis according to fibrosis stage. SVR, sustained virological response; F, fibrosis according to Metavir; SVR12, sustained virological response at week 12 after therapy; SVR24, sustained virological response at week 24 after therapy.

Figure 2. Sustained virological response rates at week 24 after therapy in the 96 cirrhotic patients. SVR24, sustained virological response at week 24 after therapy; CI, confidence interval; *IL28B*, *Interleukin 28B*; HLA, human leukocyte antigen; TW4, week 4 on therapy; MELD, Model for End-stage Liver Disease; APRI, AST to platelet ratio index; kPa, kilopascal.

Figure 13. Variations of from baseline to weeks 24 and 48 after therapy in APRI (Panels A and B) and FIB-4 (Panels C and D) from baseline to weeks 24 and 48 after therapy, in F3 and F4 long-term responders. patients who achieved viral eradication. APRI, AST to platelet ratio index; F, fibrosis according to Metavir; SVR24, sustained virological response at week 24 after therapy; SVR48, sustained virological response at week 48 after therapy.

Figure 24. Variations in of liver stiffness from baseline to weeks 24 and 48 after therapy in F3 (Panel A) and F4 patients (Panel B) who achieved viral eradication long-term responders. F, fibrosis according to Metavir; kPa, kilopascal; SVR24, sustained virological response at week 24 after therapy; SVR48, sustained virological response at week 48 after therapy.

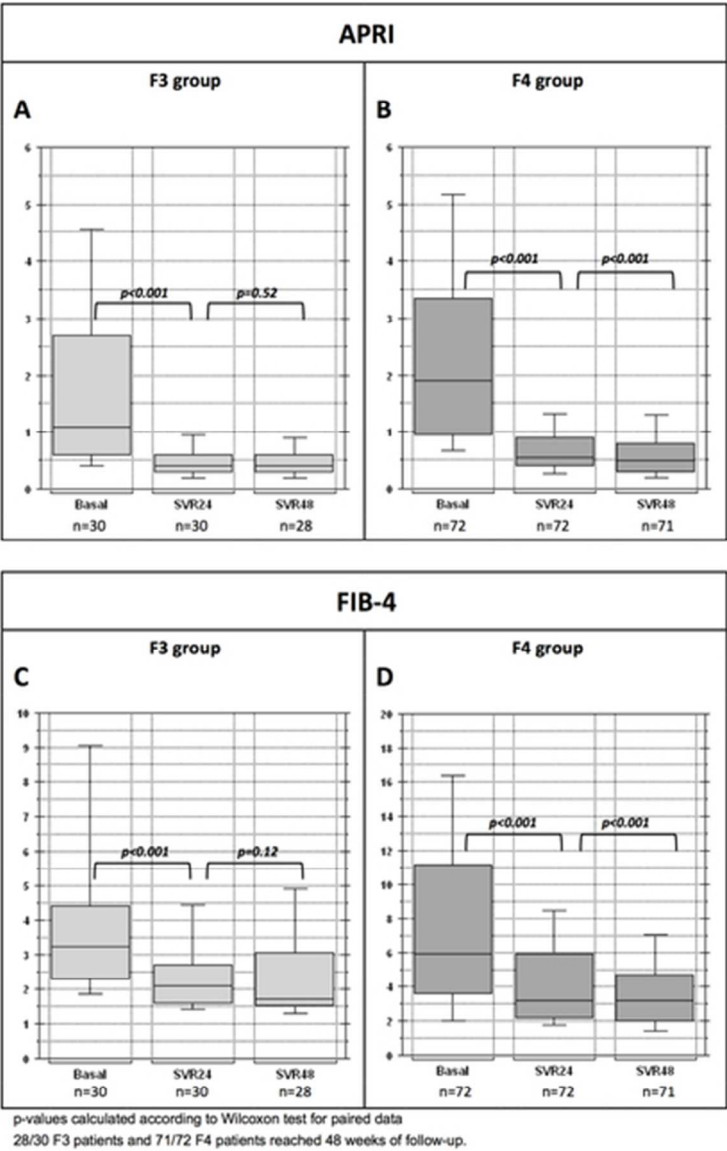


Figure 1. Variations of APRI (Panels A and B) and FIB-4 (Panels C and D) from baseline to weeks 24 and 48 after therapy, in F3 and F4 long-term responders. APRI, AST to platelet ratio index; F, fibrosis according to Metavir; SVR24, sustained virological response at week 24 after therapy; SVR48, sustained virological response at week 48 after therapy.

194x236mm (72 x 72 DPI)

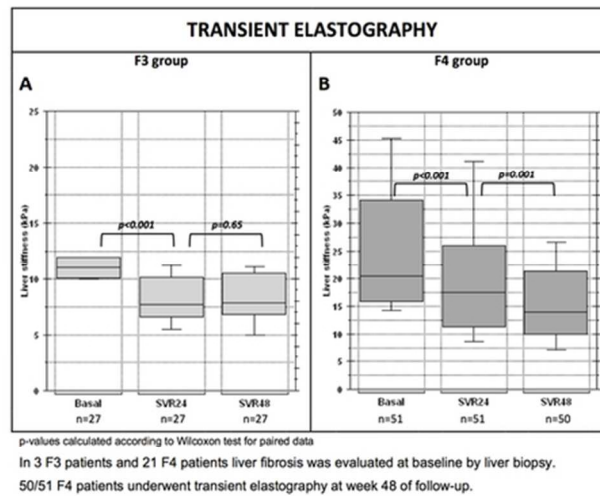


Figure 2. Variations of liver stiffness from baseline to weeks 24 and 48 after therapy in F3 (Panel A) and F4 (Panel B) long-term responders. F, fibrosis according to Metavir; kPa, kilopascal; SVR24, sustained virological response at week 24 after therapy; SVR48, sustained virological response at week 48 after therapy.

260x142mm (72 x 72 DPI)