ORIGINAL ARTICLE

The Italian compassionate use of sofosbuvir observational cohort study for the treatment of recurrent hepatitis C: clinical and virological outcomes

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SUMMARY

Direct antivirals are available for treating recurrent hepatitis C (RHC). This study reported outcomes of 424 patients with METAVIR F3–F4 RHC who were treated for 24 weeks with sofosbuvir/ribavirin and followed for 12 weeks within the Italian sofosbuvir compassionate use program. In 55 patients, daclatasvir or simeprevir were added. Child–Pugh class and model of end stage liver disease (MELD) scores were evaluated at baseline and 36 weeks after the start of therapy. The sustained viral response (SVR) was 86.7% (316/365) in patients who received sofosbuvir/ribavirin and 98.3% (58/59) in patients who received a second antiviral ($P < 0.01$). In patients treated with sofosbuvir/ribavirin, a significant difference in SVR was observed between patients diagnosed with METAVIR F4 (211/250; 84.4%), METAVIR F3 (95/105; 90.5%) and fibrosing cholestatic hepatitis (10/10; 100%) ($P = 0.049$). A significant association was found between patients who worsened from Child–Pugh class A and who experienced viral relapse (4/26 vs. 8/189, $P = 0.02$). In patients with a baseline MELD score <15, a significant association was found between maintaining a final MELD score <15 and the achievement of SVR (187/219 vs. 6/10, $P = 0.031$). This real-world study indicates that sofosbuvir/ribavirin treatment for 24 weeks was effective, and the achievement of SVR was associated with a reduced probability of developing worsening liver function.

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Introduction

Hepatitis C virus (HCV)-related end stage liver disease is the most frequent indication for liver transplantation (LT) in the US and Europe [1]. In recipients who undergo LT with active HCV replication, recurrent infection in the graft develops universally, leading to a more aggressive course compared to nonliver transplanted patients [2]. It has been demonstrated that approximately 30% of recipients with HCV recurrence develop cirrhosis of the graft within 5 years [3] and that recurrent infection is the most frequent cause of mortality and graft loss in these patients [4]. Several factors have been associated with the increased risk of severe HCV progression; however, many of these factors are not modifiable, such as donor age, pre-LT HCV viral load and HCV genotype [2]. Thus, the only factor demonstrated to have a major impact on the natural history of recurrent HCV-induced hepatitis (RHC) after LT is an effective antiviral therapy to eradicate HCV infection [5,6].

Sofosbuvir (SOF) is the first potent and pan-genotype inhibitor of the HCV NS5B polymerase that has been used in combination with ribavirin (RBV) with and without pegylated interferon to treat HCV chronic infection in phase III clinical trials [7,8]. The major advantage of SOF compared to first generation direct antiviral agents (DAAs) is a high genetic barrier and a more favourable toxicity profile; furthermore, no interactions with calcineurin inhibitors or other immunosuppressive drugs adopted in an LT setting have been demonstrated [9].

The introduction of SOF in clinical practice has taken place at different times in European countries. In Italy, the possibility of using SOF for the treatment of RHC after LT arose in 2014, through a compassionate national study.

Here, we report the real-world virological and clinical outcomes of a large cohort of HCV-positive LT patients who received, on the basis of this compassionate program, antiviral treatment for RHC with a combination of SOF plus RBV for 24 weeks.

Patients and methods

Study design

This was an observational cohort study promoted by the Italian Association for the Study of the Liver (AISF) and the Italian Society of Infectious and Tropical Diseases (SIMIT), and the study was endorsed by the Italian Medicines Agency (AIFA). Patients fulfilling the following inclusion criteria were enrolled in the Italian compassionate program of SOF (ITACOPS) for the treatment of RHC between June and December 2014: age >18 years, creatinine clearance ≥30 ml/min/1.73 m^2 (calculated by means of CKD-EPI formula) and RHC of any genotype, with significant fibrosis (METAVIR >F2) or with fibrosing cholestatic hepatitis (FCH). The fibrosis score was assessed by liver histology or by transient elastography (Fibroscan®) performed within 3 months before the start of antiviral therapy. Minimum cut-off values of liver stiffness were selected at 10 and 12 kPa to identify patients with significant (F3) fibrosis and cirrhosis, respectively, as suggested by Italian guidelines [10]. Sofosbuvir was provided by Gilead Science free of charge for the Italian National Health System. Access to the compassionate program was given after approval by a local Ethics Committee and Gilead Science, in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Treatment schedule

All patients received a combination of SOF 400 mg daily plus RBV at a starting daily dose of 1000 mg (for body weight <75 kg) or 1200 mg (for body weight ≥75 kg) for 24 weeks. Modifications to the RBV dose as well as for calcineurin and mTOR inhibitors were performed at the investigator's discretion on the basis of blood haemoglobin levels and trough serum levels of immunosuppressive drugs. According to the protocol, a second DAA could be allowed if obtained for compassionate use.

Monitoring

Patients receiving antiviral treatment were reviewed at baseline, at week 4 of treatment and 12 weeks after the end of the 24-week treatment. Clinical events and biochemical parameters, measured at local accredited laboratories, were recorded on a standardized eCRF (Ibis Informatica, Milan, Italy). HCV-RNA levels were measured either by Roche High-Pure-System/COBAS(®) TaqMan(®) v2.0 assay (LLOQ 15 IU/ml; Roche Diagnostics, Indianapolis, IN, USA) or by Abbott real-time assay (LLOQ 12 IU/ml; Abbott Molecular Inc., Des Plaines, IL, USA). Child–Pugh (CP) class and model of end stage liver disease (MELD) scores were recorded at baseline and 12 weeks after the end of antiviral treatment. Serious adverse events related to the use of SOF, as well as data concerning modification of RBV dosage, use of blood transfusions or erythropoietin, were recorded as categorical variables.

Outcome measures

The primary outcome was the achievement of sustained viral response 12 weeks after the end of treatment (SVR12), defined as undetectable serum HCV-RNA measured at the local laboratory with a lower limit of quantification of <15 IU/ml. Secondary outcomes included changes in CP class and MELD scores from baseline to 12 weeks after the end of treatment in patients with METAVIR F4.

Statistical analysis

Statistical analysis of data was performed using the BMDP dynamic statistical software package 7.0 (Statistical Solutions, Cork, Ireland). Continuous variables are presented as medians (interquartile ranges, IRQ). Categorical variables are presented as frequencies (%). Differences between categorical variables were evaluated by means of the Pearson chi-squared test or chi-squared test for linear trend when appropriate. Analysis of variance with repeated measures was used to evaluate the differences in mean values of serum creatinine during antiviral treatment. The McNemar test of symmetry was used to determine the differences in marginal frequencies. Stepwise logistic regression analysis with a forward approach was performed to identify independent predictors of SVR12; odds ratios (O.R.) and 95% confidence intervals (C.I.) were also assessed. In this analysis, all demographic and clinical variables that reached statistical significance in predicting SVR12 with $P < 0.10$ in the univariate analysis were included.

Results

Seven hundred twenty-eight patients had access to the ITACOPS compassionate program. In 170 patients, no data on antiviral response to SOF plus RBV were available, and data were available only at week 4 after the start of treatment in 134 patients. A total of 424 patients had complete data for antiviral response both at the end of treatment and 12 weeks thereafter. Among these patients, 59 (14%) received a second DAA added to SOF plus RBV: simeprevir (SMV) in 21 (5.0%) and daclatasvir (DCV) in 38 (9.0%) patients. A diagram illustrating the selection of patients included in the final evaluation is shown in Fig. 1.

This report analysed separately the 365 patients who completed 24 weeks of antiviral treatment with SOF plus RBV and 12 weeks of follow-up and the 59 patients who received a second DAA. The main demographic and clinical characteristics of the two populations are reported in Table 1. Patients treated with a second DAA were more frequently infected by HCV genotype 1a, received a lower daily dose of RBV and were less frequently immunosuppressed with cyclosporine compared to those treated with SOF plus RBV alone. No significant differences were found between the two groups in terms of severity of liver disease, as calculated by MELD scores or CP classes.

Virological outcomes

In patients treated with SOF plus RBV, overall SVR12 was achieved in 316/365 (86.7%) patients. The 49/365 (13.4%) patients who did not achieve SVR12 showed a reappearance of HCV-RNA in the serum after the end of treatment and thus were considered relapsers. A significant trend in achieving SVR12 was observed in patients starting with METAVIR F4 (211/250; 84.4%) and increasing in those with METAVIR F3 (95/105; 90.5%) and those with FCH (10/10; 100%) ($P = 0.049$). Regarding HCV genotypes, SVR12 was achieved in 238/277 (85.9%) genotype 1 patients, in 17/18 (94.4%) genotype 2 patients, in 44/52 (84.6%) genotype 3 patients and in 17/18 (94.4%) genotypes 4–6 patients. In the 250 patients with METAVIR F4, SVR12 was achieved in 154/184 (83.7%) genotype 1 patients, in 15/16 (93.7%) genotype 2 patients, in 30/37 (81.1%) genotype 3 patients and in 12/13 (92.3%) genotypes 4–6 patients. In F3 patients, SVR12 was achieved in 76/85 (89.4%) genotype 1 patients, in 1/1 (100%) genotype 2 patients, in 13/14 (92.9%) genotype 3 patients and in 5/5 (100%) genotypes 4–6 patients (Fig. 2). No significant difference in the achievement of SVR12 in patients with both METAVIR F4 and F3 was observed between genotypes 1a and 1b: 37/41 vs. 117/143 ($P = 0.198$) and 15/15 vs. 61/70 ($P = 0.142$), respectively.

SVR12 in the 59 patients who received a second DAA was achieved in 37/38 patients (97.4%) treated with DCV and in 21/21 patients (100%) treated with SMV. The only patient treated with DCV who did not achieve SVR12 was infected by HCV genotype 1a and was cirrhotic. As only one patient treated with a second DAA failed, further analyses have been performed in the 365 patients who were treated with SOF plus RBV alone.

Baseline clinical, demographic and virological factors that might predict SVR12 were investigated. Baseline factors that significantly predicted the achievement of SVR12 via univariate analysis were as follows:

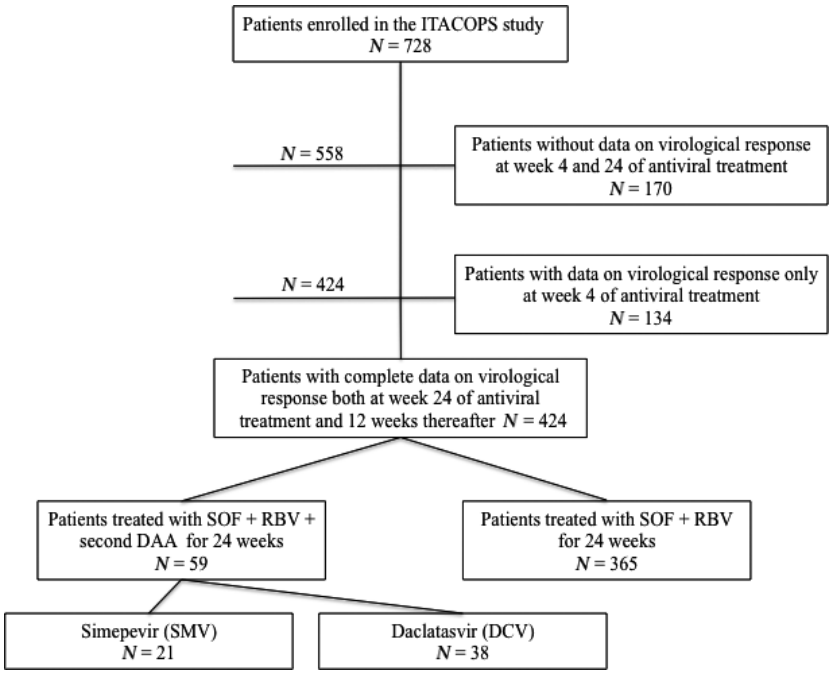

Figure 1 Diagram illustrating the selection of patients who completed 24 weeks of antiviral treatment and 12 weeks of follow-up. The numbers on the left side of the diagram refer to patients not included in the final evaluation for each of the reasons listed on the right side of the chart.

haemoglobin level >12 g/dl, albumin level >3.5 g/dl, the absence of HCV-RNA 4 weeks after the start of antiviral treatment and cyclosporine-based immunosuppression. Multivariate analysis confirmed both a haemoglobin level >12 g/dl and a serum albumin level >3.5 g/dl as independent baseline predictors of SVR12. No significant impact in predicting treatment response was associated with higher baseline HCV-RNA viral load, HCV genotype or RBV dose (Table 2). In patients with METAVIR F4, baseline haemoglobin levels >12 g/dl, platelet counts ≥100 000/mm^3, serum albumin levels >3.5 g/dl, the absence of ascites, the absence of HCV-RNA 4 weeks after the start of antiviral treatment and immunosuppressive treatment with cyclosporine were identified as predictors of SVR12 via univariate analysis. Multivariate analysis confirmed only haemoglobin levels >12 g/dl and serum albumin >3.5 g/dl as baseline independent predictors of SVR12 (Table 3). Comparing the baseline clinical, demographic and virological characteristics of patients who received immunosuppressive therapy with tacrolimus or cyclosporine, no significant differences were found concerning age, gender, severity of liver disease and response to antiviral therapy at week 4. In contrast, patients treated with cyclosporine showed higher baseline HCV-RNA serum levels compared to those treated with tacrolimus (Table 4).

Table 5 shows the main baseline clinical, demographic and virological parameters of 365 patients who have been fully analysed compared to the 134 patients for whom data regarding viral response were available only at week 4. No significant differences were found between the two groups.

Functional outcomes

An analysis of functional outcomes was performed only in patients with METAVIR F4 ($N = 250$). The differences in CP classes and MELD scores from baseline to SVR12 as well as the changes in serum levels of albumin were determined both in patients who achieved SVR12 and in those who relapsed. Considering all 250 patients, CP class calculated 12 weeks after the end of antiviral treatment compared to baseline improved in 35 patients (14.0%), remained stable in 207 patients (82.8%) and worsened in eight patients (3.2%) ($P < 0.001$ by McNemar test of symmetry). Considering patients who achieved SVR12 ($N = 211$), CP class improved in 30 patients (14.2%), remained stable in 177 patients (83.8%) and worsened in four patients (1.8%) ($P < 0.001$ by McNemar test of symmetry). Among the 39 patients who relapsed, CP class improved in five patients (12.8%), remained stable in 30 patients (76.9%) and worsened in four patients (10.3%) ($P = 0.739$ by McNemar test of symmetry; Table 6). A significant association was found between patients who worsened from CP class A and patients who experienced viral relapse (4/26 vs. 8/189, $P = 0.02$).

Table 1. Baseline demographic, clinical and virological characteristics of the studied population (N = 424).

Baseline characteristics	Patients who received SOF/R plus a second DAA (N = 59)	Patients who received SOF/R (N = 365)	P
Age, years	58.7 ± 1.1	60.6 ± 0.43	0.101
Male gender	49 (83.1)	292 (80.0)	0.584
Body weight, kg	71.8 ± 1.5	73.5 ± 0.6	0.300
HCV genotypes			
1a	18 (30.5)	59 (16.2)	<0.001
1b	33 (55.9)	218 (59.8)	0.582
2	0 (0.0)	18 (4.9)	0.081
3	4 (6.8)	52 (14.2)	0.116
4–6	4 (6.8)	18 (4.9)	0.553
HCV-RNA IU/ml $\times 10^7$	5.8 ± 1.7	4.2 ± 0.4	0.252
MELD score*	10.4 ± 0.5	10.0 ± 0.2	0.403
A	31	189	0.596
CP class*			
B	7	56	0.580
C	0	5	0.379
RBV mg/die	461 ± 45	767 ± 14	<0.001
Absent	27	183	0.781
Ascites*			
Moderate	11	55	0.342
Severe	0	12	0.168
Absent	34	222	0.902
EPS*			
Grade I	3	28	0.540
Grade II–III	1	0	0.010
Bilirubin mg/dl	3.0 ± 0.6	5.7 ± 0.4	0.004
INR	1.15 ± 0.03	1.09 ± 0.01	0.045
Albumin g/dl	3.9 ± 0.07	3.9 ± 0.03	0.581
Creatinine mg/dl	1.1 ± 0.04	1.1 ± 0.02	0.567
Haemoglobin g/dl	13.0 ± 0.2	13.1 ± 0.1	0.543
AST IU/l	94 ± 10	86 ± 4	0.400
ALT IU/l	88 ± 9	83 ± 4	0.631
Platelets $\times 10^3/mm^3$	100 ± 7	121 ± 4	0.041
Cyclosporine-based IS	15 (25.4)	155 (42.5)	0.013
HCC	3 (5.1)	33 (9.0)	0.312
FCH	1 (1.7)	4 (11.1)	0.692
METAVIR			
3	19 (32.8)	105 (29.2)	0.590
4	38 (65.5)	250 (69.6)	0.533
Naive to antivirals	14 (23.7)	138 (37.8)	0.036

MELD, model of end stage liver disease; CP, Child–Pugh; RBV, ribavirin; EPS, hepatic encephalopathy; INR, international normalized ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase, IS, immunosuppression; HCC, hepatocellular carcinoma; FCH, fibrosing cholestatic hepatitis.

Patients are divided into two groups: those who were treated with sofosbuvir (SOF) plus ribavirin (RBV) and a second direct antiviral agent (DAA) and those who received SOF/RBV alone for 24 weeks. Continuous variables are presented as the mean ± standard error of the mean (SEM), and categorical variables are presented as frequencies (%).

*Calculated in patients with METAVIR F4 (N = 288).

For patients with METAVIR F4, compared to baseline, MELD scores 12 weeks after the end of treatment improved in 103 patients (41.2%), remained stable in 58 patients (23.2%) and worsened in 89 patients (35.6%) (Fig. 3). In patients with baseline MELD scores ≥15 (N = 21), the MELD score calculated 12 weeks after the end of treatment was <15 and ≥15 in 12 patients (57.1%) and nine patients (42.9%), respectively.

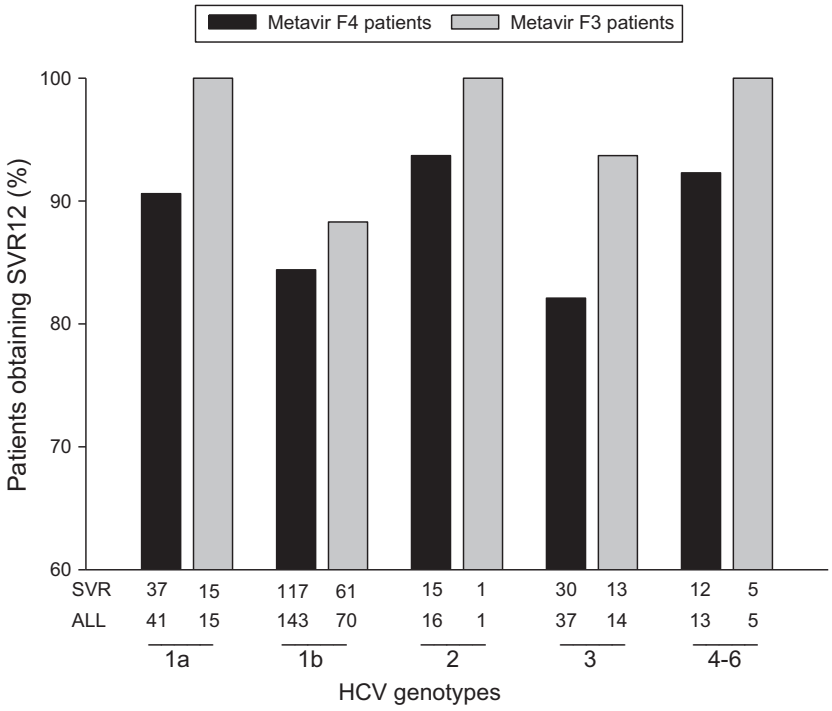

Figure 2 Rates of sustained virological response 12 weeks after the end of treatment sustained viral response (SVR12) in relation to hepatitis C virus (HCV) genotype and the degree of liver fibrosis (METAVIR F3 vs. F4).

Table 2. Univariate and multivariate analyses of baseline demographic and clinical characteristics of HCV-positive liver transplantation (LT) recipients treated with sofosbuvir/ribavirin for 24 weeks (*N* = 365) in relation to the achievement of SVR12.

	Univariate analysis			Multivariate analysis		
Baseline parameters	SVR12 NO (*N* = 49)	SVR12 YES (*N* = 316)	*P*	O.R.	95% C.I.	*P*
Age >60 years	23 (46.9)	172 (54.4)	0.328			
Male gender	40 (81.6)	252 (79.7)	0.759			
Body weight >75 kg	25 (51.0)	126 (39.9)	0.140	0.54	0.29–1.02	0.056
AST >40 UI/l	39 (79.6)	232 (73.4)	0.358			
ALT >40 IU/l	38 (77.6)	238 (75.3)	0.735			
Haemoglobin >12 g/dl	26 (53.1)	227 (71.8)	0.008	2.13	1.11–4.07	0.043
Platelets >100 000/mm^3	22 (44.9)	188 (59.5)	0.054			
Bilirubin >2.0 mg/dl	23 (46.9)	156 (49.4)	0.752			
Creatinine >1.5 mg/dl	5 (10.2)	28 (8.9)	0.760			
HCV-RNA >1 × 10^6 IU/ml	33 (67.3)	218 (69.0)	0.818			
HCV genotype 2 versus others	1 (2.0)	17 (5.4)	0.315			
Albumin >3.5 g/dl	27 (55.1)	250 (79.1)	<0.001	2.80	1.47–5.33	0.001
METAVIR F4	39 (79.6)	211 (66.8)	0.072			
RBV die >500 mg	37 (75.5)	245 (77.5)	0.753			
HCV-RNA-negative at week 4 of treatment	20 (40.8)	185 (58.5)	0.020			
Cyclosporine-based IS	14 (28.6)	141 (44.6)	0.035			
HCC yes	4 (8.2)	29 (9.2)	0.818			
Naïve to antiviral treatment	24 (49.0)	114 (36.1)	0.083			

AST, aspartate aminotransferase; ALT, alanine aminotransferase; RBV, ribavirin; IS, immunosuppression; HCC, hepatocellular carcinoma.

Univariate and multivariate analyses were performed by means of Pearson chi-squared test and stepwise logistic regression. Odds ratios (O.R.) and 95% confidence intervals (C.I.) are also provided.

Table 3. Univariate and multivariate analyses of baseline demographic and clinical characteristics of METAVIR F4 HCV-positive liver transplantation (LT) recipients treated with sofosbuvir/ribavirin for 24 weeks ($N = 250$) in relation to the achievement of SVR12.

	Univariate analysis			Multivariate analysis		
Baseline parameters	SVR12 NO ($N = 39$)	SVR12 YES ($N = 211$)	P	O.R.	95% C.I.	P
Age >60 years	19 (48.7)	119 (56.4)	0.376			
Male gender	32 (82.1)	166 (78.7)	0.633			
Body weight >75 kg	18 (46.2)	90 (42.7)	0.685			
AST >40 UI/l	32 (82.1)	160 (75.8)	0.400			
ALT >40 IU/l	30 (76.9)	157 (74.4)	0.740			
Haemoglobin >12 g/dl	19 (48.7)	155 (73.5)	0.002	2.45	1.19–5.05	0.016
Platelets >100 000/mm^3	14 (35.9)	118 (55.9)	0.021			
Bilirubin >2.0 mg/dl	20 (51.3)	110 (52.1)	0.922			
Creatinine >1.5 mg/dl	4 (10.3)	18 (8.5)	0.727			
HCV-RNA >1 × 10^6 IU/ml	25 (64.1)	147 (69.7)	0.491			
HCV genotype 2 versus others	1 (2.6)	15 (7.1)	0.287			
Albumin >3.5 g/dl	18 (46.2)	156 (73.9)	<0.001	2.85	1.39–5.87	0.001
Ascites absent	21 (53.8)	162 (76.8)	0.003			
EPS absent	35 (89.7)	187 (88.6)	0.839			
RBV die >500 mg	30 (76.9)	164 (77.7)	0.912			
HCV-RNA-negative at week 4 of treatment	15 (38.5)	124 (58.8)	0.019			
Cyclosporine-based IS	11 (28.2)	99 (46.9)	0.030			
CP						
A	26 (66.7)	163 (77.3)	0.179			
B	11 (28.2)	45 (21.3)				
C	2 (5.1)	3 (1.4)				
MELD score >10	18 (46.2)	70 (33.2)	0.119			
HCC yes	4 (10.3)	14 (6.6)	0.421			
Naïve to antiviral treatment	19 (48.7)	85 (40.3)	0.326			

AST, aspartate aminotransferase; ALT, alanine aminotransferase; RBV, ribavirin; IS, immunosuppression; EPS, hepatic encephalopathy; CP, Child–Pugh; MELD, model of end stage liver disease; HCC, hepatocellular carcinoma.

Univariate and multivariate analyses were performed by means of Pearson chi-squared test and stepwise logistic regression. Odds ratios (O.R.) and 95% confidence intervals (C.I.) are also provided.

In the 229 patients with baseline MELD scores <15, the MELD score calculated 12 weeks after the end of treatment was <15 in 219 patients (95.6%) and ≥15 in 10 patients (4.4%). In patients with baseline MELD scores ≥15, no association was observed between reaching a final MELD score <15 and the achievement of SVR12 (10/12 vs. 8/9, $P = 0.718$). In contrast, in patients with baseline MELD scores <15, a significant association was found between maintaining a final MELD score <15 and the achievement of SVR12 (187/219 vs. 6/10, $P = 0.031$) (Fig. 4).

Treatment safety

No major side effects were reported regarding the use of SOF. In 13/365 (3.6%) patients, the investigators modified the dosage of calcineurin inhibitors during antiviral treatment. Mean (±SD) creatinine serum levels did not change significantly from baseline (1.13 ± 0.32 mg/dl) to week 4 (1.12 ± 0.31 mg/dl) and week 24 (1.13 ± 0.32 mg/dl, $P = 0.602$). As expected, the major side effect of RBV administration was the development of anaemia, which was judged by the investigators to be clinically sufficient to require RBV dose reduction in 94 cases (25.8%), the administration of erythropoietin in 87/365 cases (23.8%) and blood transfusion in 17/365 cases (4.6%). No significant difference in the need for RBV dose reduction was found between METAVIR F4 and the remaining patients (67/250 vs. 27/115, $P = 0.500$). Furthermore, no differences were found in the number of patients who required RBV dose reduction and had a baseline creatinine value >1.3 mg/dl (18/77 vs. 76/288, $P = 0.591$). No episodes of acute cellular rejection were recorded.

Table 4. Comparisons between the main baseline demographic, clinical and virologic characteristics of patients treated with sofosbuvir/ribavirin for 24 weeks (*N* = 365) in relation to the method of immunosuppression adopted (tacrolimus or cyclosporine).

	Tacrolimus	Cyclosporine	*P*
Baseline parameters	*N* = 210	*N* = 155	
Age >60 years	111 (52.9%)	84 (54.2%)	0.800
Male gender	173 (82.4%)	119 (76.8%)	0.186
HCV-RNA >1 × 10^6 IU/ml	127 (60.5%)	124 (80.0%)	0.0001
METAVIR F4	140 (66.7%)	110 (71.0%)	0.382
Week 4 response	119 (56.7%)	84 (54.2%)	0.638
Patients with METAVIR F3	*N* = 70	*N* = 45	
Week 4 response	40 (57.1%)	25 (55.6%)	0.867
Patients with METAVIR F4	*N* = 140	*N* = 110	
Week 4 response	79 (56.4%)	60 (54.5%)	0.766

The frequency of HCV-RNA negativity recorded at week 4 after the start of antiviral therapy (week 4 response) is analysed separately in patients with METAVIR F3 and MEATVIR F4. The analyses were performed by means of Pearson chi-squared test.

Table 5. Comparisons between the main baseline demographic, clinical and virological characteristics of patients treated with sofosbuvir/ribavirin for whom data regarding sustained viral response 12 weeks after the end of treatment (SVR12) were available, and patients for whom data regarding viral response were available only 4 weeks after the start of therapy.

Baseline parameters	Patients with complete data on viral response *N* = 365 (%)	Patients with viral response recorded only at week 4 *N* = 134 (%)	*P*
Age >60 years	195 (53.4)	61 (45.5)	0.117
Male gender	292 (80.0)	104 (77.6)	0.559
HCV genotype 2 versus others	18 (4.9)	5 (3.7)	0.571
METAVIR F4	250 (68.5)	81 (60.4)	0.091
Week 4 response	205 (56.2)	69 (51.5)	0.352

Analyses were performed by means of Pearson chi-squared test.

Table 6. Child–Pugh (CP) class modifications in patients with METAVIR F4 evaluated 12 weeks after the end of antiviral treatment compared to baseline.

	Baseline CP class in patients with METAVIR F4		
	A	B	C
	189 (75.6%)	56 (22.4%)	5 (2.0%)
12 week CP class	All patients (*N* = 250)*		
A (*N* = 216; 86.4%)	181 (95.8%)	33 (58.9%)	2 (40.0%)
B (*N* = 30; 12.0%)	7 (3.7%)	23 (41.1%)	0 (0.0%)
C (*N* = 4; 1.6%)	1 (0.5%)	0 (0.0%)	3 (60.0%)
	Patients with SVR12 (*N* = 211; 84.4%)*		
A (*N* = 220; 88.7%)	159 (97.6%)	28 (62.2%)	2 (66.7%)
B (*N* = 24; 9.7%)	3 (1.8%)	17 (37.8%)	0 (0.0%)
C (*N* = 4; 1.6%)	1 (0.6%)	0 (0.0%)	1 (33.6%)
	Patients without SVR12 (*N* = 39; 15.6%)		
A (*N* = 28; 70.0%)	22 (84.6%)	5 (45.5%)	0 (0.0%)
B (*N* = 10; 25.0%)	4 (15.4%)	6 (54.5%)	0 (0.0%)
C (*N* = 2; 5.0%)	0 (0.0%)	0 (0.0%)	2 (100%)

Data presented refer to all patients, patients who achieved sustained viral response 12 weeks after the end of treatment (SVR12) and patients who relapsed.

*$P < 0.001$ by McNemar test of symmetry.

Discussion

To our knowledge, this report comprises the largest series of LT recipients with severe RHC (METAVIR F3–4 and FCH) treated with SOF-based antiviral therapy. The most important results of this study are that antiviral therapy with SOF plus RBV for 24 weeks led to SVR12 in more than 85% of cases, and that a significant difference in obtaining higher rates of SVR12 was demonstrated between patients with FCH, METAVIR F3 and METAVIR F4.

SVR12 rates in patients with FCH were 100%, in agreement with a French study in which SOF-based therapy induced SVR12 in 97% of patients with FCH [11]. The negative effect of baseline cirrhosis on the probability of achieving SVR has been well-documented in patients treated with interferon plus first generation DAAs [12]. This negative effect has become less

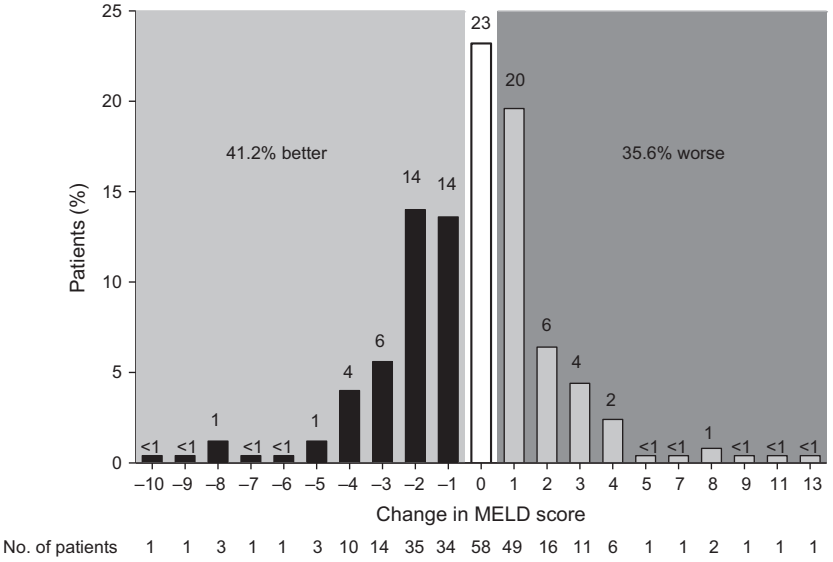

Figure 3 Changes in model of end stage liver disease (MELD) score calculated 12 weeks after the end of antiviral therapy compared to baseline. Data refer to patients with METAVIR F4.

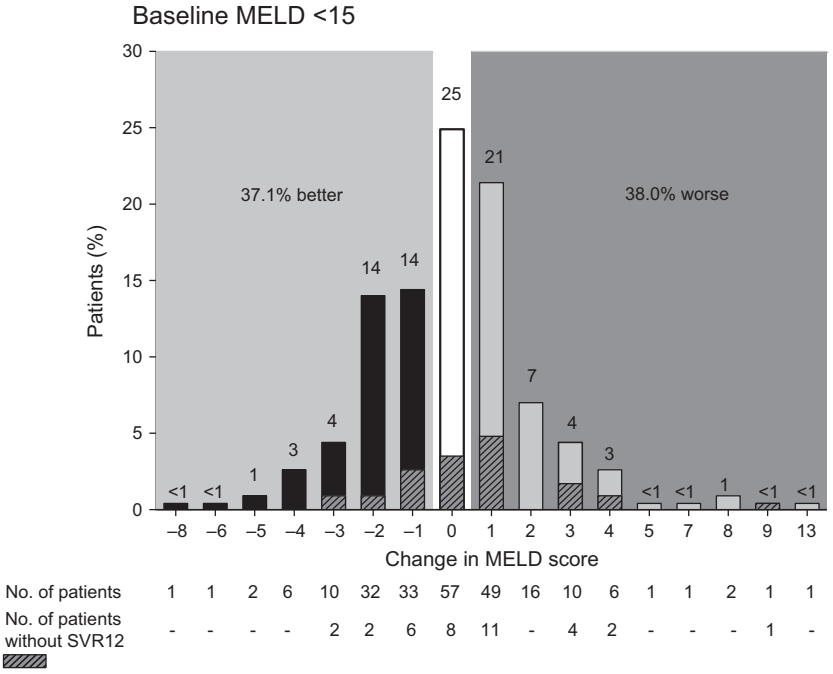

Figure 4 Changes in model of end stage liver disease (MELD) score calculated 12 weeks after the end of antiviral treatment compared to baseline in relation to the achievement of sustained viral response 12 weeks after the end of treatment sustained viral response (SVR12). Data refer to patients with baseline MELD scores <15.

pronounced with the availability of more recent treatment combinations of SOF plus ledipasvir (LDV). In the SOLAR-1 [13] and SOLAR-2 [14] clinical trials, SVR12 was achieved in 96–98% of patients without cirrhosis or with compensated cirrhosis. Considering only the 59 patients treated in the present study with SOF and RBV plus DCV or SMV, SVR12 was achieved in 97.4% of cases, confirming the results obtained in the SOLAR studies. It is important to note, however, that even when adopting the antiviral combination based on SOF/LDV plus RBV, the SVR12 rates in patients with moderate liver impairment were 85–88%, and the rates were 60–75% in patients with severe liver impairment at baseline. These data confirm that the addition of a second DAA to SOF does not increase the SVR12 rates in patients with significant liver impairment and suggests that early antiviral treatment for RHC offers a clear advantage over waiting until a patient develops decompensated cirrhosis.

The impact of baseline liver function in conditioning the antiviral response to SOF-based therapy is of particular interest. Through univariate analysis, baseline predictors of the achievement of SVR12 in our patients with METAVIR F4 were haemoglobin levels >12 g/dl,

serum albumin levels >3.5 g/dl, platelet counts >100 000/mm^3, the absence of ascites, cyclosporine-based immunosuppressive therapy and the absence of HCV-RNA at week 4 after the start of treatment. In a recent large real-world study involving SOF-based treatments in patients with advanced liver disease, patients with serum albumin levels <3.5 g/dl and aged ≥65 years had the lowest odds of deriving functional benefit from antiviral treatment [15], confirming that impaired synthetic liver function remains an independent negative predictor of SVR even when adopting antiviral treatment schemes including a second DAA with SOF plus RBV.

Concerning the HCV-RNA kinetics during treatment, in our series, the achievement of HCV-RNA negativity at week 4 of treatment was associated with the achievement of SVR12 only in the univariate analysis. The usefulness of HCV-RNA decline at week 2 of therapy to predict relapse in patients treated with SOF plus RBV has been demonstrated in 33 HCV genotype 3 nonliver transplanted patients [16]. Approximately 45% of patients became HCV-RNA negative at week 4 in our series, which was comparable to that reported by Maasoumy *et al.* [16], whereas no differences were found in the percentages of week 4 responses between HCV genotype 3 patients compared to other genotypes for either the METAVIR F4 or F3 recipients (data not shown). The SVR12 rate in HCV genotype 3 patients in our series was higher than that reported by Maasoumy *et al.* [16], and the week 4 response was not associated with the achievement of SVR12. These differences could be explained by the fact that in our series, a higher number of genotype 3 patients were enrolled and that patients had a different clinical condition due to the presence of RHC.

Although our study was not designed to determine whether cyclosporine could be superior to tacrolimus in conditioning the antiviral response, in univariate analysis the use of cyclosporine was associated with a better likelihood of achieving SVR12. This finding is very interesting because although the potential benefit of cyclosporine in increasing SVR has been previously demonstrated in liver transplanted patients treated with interferon-based regimens [17], it has not been demonstrated using DAAs. The influence of cyclosporine in increasing rates of SVR12 could be explained by its additive direct antiviral effect against HCV *in vitro* [18]. This effect could explain the observation that although baseline HCV-RNA levels were significantly higher in patients receiving cyclosporine compared to those receiving tacrolimus in our series, this did not have a negative effect either in HCV-RNA kinetics at week 4 or in SVR12. Another potential effect of cyclosporine in determining SVR could be related to the fact that the simultaneous administration of cyclosporine and sofosbuvir is associated with an increase in plasma levels of sofosbuvir [9] which, although not suggesting a dose modification, may be sufficient to increase its antiviral action, especially if used as a single direct antiviral drug as in our study.

The clinical significance of SVR12 achievement in patients with advanced liver disease remains a major clinical question. Approximately 14% of patients improved their CP class, with 33/56 patients progressing from CP Class B to Class A and 2/5 patients progressing from CP class C to CP class A. Moreover, the proportion of patients who remained in CP class A was significantly higher in patients who achieved SVR12 compared to patients who relapsed (159/163 vs. 22/26, $P = 0.002$). These results suggest that the resumption of viral replication in cirrhotic patients adversely affects hepatic synthetic function even in a short period of time, such as the follow-up in our study.

Our results deriving from the MELD score modifications are also clinically interesting. Although an improvement of the MELD score was achieved in over 40% of patients, it worsened in 35% of cases, indicating that viral eradication does not prevent clinical deterioration, at least in the short term, in a subgroup of patients. Similar results were observed in the SOLAR-I study [13], in which MELD scores worsened in approximately 25% of cases despite the achievement of SVR. These findings raise the question of identification of the degree of liver disease severity beyond which the clinical benefit of viral eradication is no longer likely—the so-called 'point of no return'. In a large real-world study [15] of patients with decompensated cirrhosis treated with SOF and RBV plus LDV or DCV, compared to an untreated control group, the proportion of patients with at least one decompensating event during the study period was reduced, apart from a subgroup of patients with baseline MELD scores ≥15. Interestingly, in patients with baseline MELD scores ≥15, the achievement of SVR12 was not associated with a significant improvement in liver function in approximately half of the patients in our study.

The most common adverse event recorded in our series was RBV-induced anaemia, which required RBV dose reduction or further treatment in approximately 30% of cases. In previous reports [19,20], the development of anaemia was not prevented by starting with low doses of RBV, suggesting that anaemia was not strictly RBV dose-related. As RBV is eliminated by the

kidneys, renal function represents an important factor in conditioning the development of RBV-induced anaemia. Nevertheless, in our series, we have not observed a significant correlation between renal function and the degree of anaemia. It is important to note that our patients showed a potential impairment of renal function in less than 10% of cases.

As RBV and SOF did not show significant drug-drug interactions with calcineurin and mTOR inhibitors [21], no major interactions between antiviral treatment and calcineurin inhibitors were reported in our series, as demonstrated by the low percentage of patients in whom it was necessary to change the dosages of immunosuppressive agents.

Our study has several limitations. SOF plus RBV combination is currently considered suboptimal, as it has been replaced for the treatment of RHC by the combination of SOF plus an NS5A inhibitor, often without RBV [22]. However, it should be noted that SOF plus RBV combination represents a real, significant innovation in the treatment of RHC, as demonstrated in this study in which the largest number of patients ever reported in the literature has been evaluated. The SVR12 rate was particularly high and very similar to the most recent therapeutic regimens, especially in patients with less severe RHC. The small number of patients who failed to achieve SVR12 was primarily infected by HCV genotype 3, which is still the most difficult HCV genotype to treat with the newest antiviral combinations. Furthermore, our study permitted the determination of the safety of SOF-based treatments in LT patients.

A second limitation is related to the relatively short period of follow-up, which does not permit us to provide more detailed data on the clinical outcomes after HCV eradication in terms of patient and graft survival and, more importantly, in the potential further long-term improvement of liver function. Furthermore, only patients who completed antiviral treatment and had sufficient follow-up to determine the SVR12 have been included; thus, we were unable to highlight the rate of premature discontinuation of antiviral treatment. However, comparing patients who had complete virological data with those who had virological data recorded only at week 4 of antiviral treatment, no significant differences in terms of demographic, clinical and virological characteristics were recorded. This leads us to hypothesize that even in patients where data for the final analysis were not available, the outcomes were likely similar to those that were reported.

In conclusion, this large real-world study indicates that SOF plus RBV for 24 weeks was an effective treatment, particularly in recipients with less severe RHC. The achievement of SVR12 was associated with both a significant clinical improvement in a large proportion of patients and a reduced probability of worsening liver function, compared to patients with viral relapse.

Authorship

PC and PT: collected data, performed statistical analysis and wrote the paper. CM, GC, AR, MRT, RL, GP, IL, GP, FPR, CC, SM: collected data and approved the final manuscript. MM, SM, LP, SP, PR, MPT: contributed to the conception of the work, edited and approved the manuscript.

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Conflicts of interest

Paola Carrai *(advisory board for Gilead Sciences and Janssen),* Guido Piai (*advisory board for Abbvie*), Silvia Martini *(advisory board for Gilead Sciences)*, Pierluigi Toniutto (speaking bureau for Gilead Sciences, Merck-Sharp & Dome, Janssen, Bristol-Meyer-Squibb, Abbvie, Kedrion).

Cristina Morelli, Gabriella Cordone, Antonietta Romano, Mariarosa Tamé, Raffaella Lionetti, Giada Petrosi, Ilaria Lenci, Francesco Paolo Russo and Carmine Coppola have no conflicts of interests.

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APPENDIX

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