New Microbiologica, 42, 4, 189-196, 2019, ISN 1121-7138

FULL PAPER

Efficacy, Safety, and Predictors of Direct-acting antivirals in Hepatitis C Virus Patients with Heterogeneous Liver Diseases

Vanessa De Pace[1], Maria Cristina Morelli[1], Matteo Ravaioli[1], Fabrizio Maggi[2], Silvia Galli[3], Vittoria Vero[4], Maria Carla Re[3], Matteo Cescon[1], Mauro Pistello[2]

[1]General and Transplant Surgery Unit, Department of Medical and Surgical Sciences, University of Bologna Sant'Orsola-Malpighi Hospital, Bologna, Italy;
[2]Virology Unit, Pisa University Hospital; Virology Section and Retrovirus Center, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy;
[3]Microbiology and Virology Unit, Department of Experimental Diagnostic and Specialty Medicine, University of Bologna and Sant'Orsola-Malpighi Hospital, Bologna, Italy;
[4]End-stage Liver Disease Unit, Department of Medical and Surgical Sciences, University of Bologna and Sant'Orsola-Malpighi Hospital, Bologna, Italy

SUMMARY

Safety, efficacy, and predictor factors of sustained-virological-response after 24 weeks of new direct-acting antivirals were evaluated in hepatitis C virus patients with different stages of hepatic disease.
260 patients, median age 60 years, of whom 48.1% cirrhotics, 17.7% liver transplant recipients, and 45.7% naïve were treated with Sofosbuvir+Ribavirine, Sofosbuvir+Simeprevir±Ribavirine, Sofosbuvir+Daclatasvir±Ribavirine, Sofosbuvir+Ledispavir±Ribavirine, Ombitasvir/Paritaprevir/Ritonavir+Ribavirine and Ombitasvir/Paritaprevir/Ritonavir+Dasabuvir±Ribavirine. Therapy outcomes, hematochemical parameters, viral replication, genotype, and resistance-associated-mutations were analyzed retrospectively.
Sustained virological response was 90.4% in the whole population, 83.2% in cirrhotics, 85% in patients with previous virological failure, 93.6% in patients >60 years, and 95.6% in liver transplant recipients. SVR24 for each drug regimen was 75% Sofosbuvir+Ribavirine, 80.4% Sofosbuvir+Simeprevir±Ribavirine, 94.3% Sofosbuvir+Daclatasvir±Ribavirine, 98.7% Sofosbuvir+Ledispavir±Ribavirine, 100% Ombitasvir/Paritaprevir/Ritonavir+Ribavirine and Ombitasvir/Paritaprevir/Ritonavir+Dasabuvir±Ribavirine. The highest sustained virological response rates were obtained with genotype-1b (95.9%). Twenty-five patients, mostly cirrhotics or suffering from severe liver complications, manifested relapse (84%), breakthrough (12%), or non-response (4%). Mild side effects were observed in 41.1% of patients. Model-for-End-Liver-Disease score <10 and alanine aminotransferase ≤20 U/L at week 8 of therapy proved positive predictors of sustained virological response.
Direct-acting antiviral therapy is efficacious and safe even in patients with advanced liver disease and/or previous virological failure; Model-for-End-Liver-Disease <10 and alanine aminotransferase reduction during therapy were found to be reliable predicting markers of sustained-virological-response.

Received April 4, 2019 *Accepted* September 15, 2019

INTRODUCTION

Hepatitis C virus (HCV) infection affects about 1% of the world's population and, since there is no vaccine and only 20% of total patients are aware of their infectious status, the number of HCV infections is still increasing; 1.74 million were reported in 2015 alone (Polaris Observatory HCV Collaborators, 2017). The development of effective direct-acting antivirals (DAA) prompted the Global Health Sector Strategy to launch a campaign to eradicate infection by extending HCV treatment to all infected individuals (WHO - Global Hepatitis Report, 2017). Even if eradication is not achieved, DAA therapy has a beneficial effect in slowing disease progression, lowering the risk of developing hepatocellular carcinoma (HCC), reducing liver damage and halting viral transmission (European Association for the Study of the Liver, 2017). Since the advent of the first HCV drugs, therapy has been aimed at obtaining high levels of sustained virological response (SVR), beginning in 1996, when interferon (IFN)-alpha was first used with an SVR of 8-21% (Lindsay KL, 1997). With the first DAAs, boceprevir and telaprevir, administered before or after pegylated IFN plus RBV, SVR rose to 60-70% in 2011 (Bacon BR *et al.*, 2011; Zeuzem S *et al.*, 2011), and climbed to >90% with new-generation, IFN-free, DAAs in 2014 (Banerjee D and Reddy KR, 2016; Werner CR *et al.*, 2016; Cheinquer H *et al.*, 2017). The most-used DAAs target the following HCV proteins: sofosbuvir (SOF) and dasabuvir (DSV): NS5B polymerase; simeprevir (SMV), paritaprevir

Key words:
Hepatitis C Virus (HCV), anti-HCV Direct-Acting Antivirals (DAA), combination therapy, viral resistance.

Corresponding author:
Dr. Vanessa De Pace
E-mail: vanessa.depace@hotmail.it, vanessa.depace@unibo.it

(PTV) and grazoprevir: protease; daclatasvir (DCV), ledipasvir (LDV), ombitasvir (OBV), velpatasvir, and elbasvir: NS5A (Myers RP *et al*., 2015). Combined DAA regimens ensure high SVR rates, a high genetic barrier to resistance, short treatment cycles, minimal adverse events, and good tolerability (Banerjee D and Reddy KR, 2016).
Several studies reported SVR rates and side effects of DAAs in clinical trials using homogeneous cohorts with the same level of infection/disease and naïve status, which are optimal study models but far from reality. In this study, the safety, efficacy and early predictors of SVR to DAA treatment were examined in patients with different stages of liver disease and/or a failed positive response to previous antiviral therapy.

MATERIALS AND METHODS

Ethics statement

This study was conducted in accordance with the Declaration of Helsinki, Regional Ethics Committee, Good Clinical Practice guidelines, and local regulatory requirements. All patients were informed and gave their consent to collect demographic, clinical and virological data, and biological samples.

Patient population

In January 2015 and December 2016, 368 HCV chronic patients were treated with different therapeutic regimens at the End-stage Liver Disease Unit of Sant'Orsola-Malpighi Hospital. Of these, 260 patients with at least 24-week follow-up post-therapy were included in this retrospective observational study. Enrollment was declined for 108 patients who were under antiviral treatment during the cases study inclusion (20.4%, 75/368), without clinical data and therapy adherence (1.1%, 4/368), subjected to compassionate use of DAAs (5.4%, 20/368) or who died of hepatic failure, sepsis/septic shock or accidental events (2.4%, 9/368). Demographic, clinical and virological data of the study population are shown by *Table 1*.
Patients were selected for DAA-based therapy after (1) hepatological assessment through liver biopsy or fibroscan, (2) evaluation of hepatic and renal functions by examining baseline laboratory parameters, (3) investigation of infection status by HCV RNA quantification and genotyping. The type of DAA regimen and the relative eligible patients were defined based on:

1. DAA approved and available at the beginning of treatment.
2. HCV genotype.
3. Stage of liver disease (e.g., high or low grade of fibrosis).
4. Hepatic complications (e.g., ascites, esophageal varices, portal hypertension).
5. Comorbidities.
6. Economic factors.
7. European Association for the Study of Liver 2015 recommendations (European Association for Study of Liver, 2015).

For each DAA combination, week ranges of treatment, median duration of treatment (weeks), and number of enrolled patients were as follows: SOF+RBV (12-48 weeks, 16 weeks: 48); SOF+SMV±RBV (12-16 weeks, 12 weeks: 51); SOF+DCV±RBV (12-24 weeks, 12 weeks: 35), SOF+LDV±RBV (12-24 weeks, 12 weeks: 77), OBV/PTV/ritonavir (OBV/PTV/r)+RBV (12-24 weeks, 20 weeks: 4), and OBV/PTV/r+DSV±RBV (12-24 weeks, 12 weeks: 45). A detailed description of the patients enrolled for each treatment is provided in the Supplementary Material.

Table 1 - *Clinical and virological characteristics of study population.*

Patients	*260*
Male	177 (68.1%)
Age (yr)	60 (53-71)
BMI >30	29 (11.2%)
Cirrhosis	125 (48.1%)
Fibrosis 3	43 (16.5%)
Fibrosis 0-2	92 (35.4%)
Liver complications	
Ascites	29 (11.2%)
Encephalopathy	15 (5.8%)
Esophageal varices	65 (25.0%)
Portal hypertension	38 (14.6%)
HCC	56 (21.5%)
Extra-liver complications	
Cryoglobulinemia	23 (8.9%)
Lymphoma	6 (2.3%)
OLT	46 (17.7%)
Awaiting OLT	16 (6.2%)
Genotype HCV	
1a	43 (16.5 %)
1b	122 (47%)
2	35 (13.4%)
3	36 (13.8%)
4	24 (9.3%)
Baseline HCV RNA (UI/mL)	1,352,207 (416,166-3,073,934)
Cirrhotics	1,049,768 (232,327-2,365,060)*
Non-cirrhotics	1,683,740 (676,015-3,575,355)*
Naïve	130 (50.0%)
Previous antiviral therapy	
IFN	19 (14.6%)
IFN+RBV	104 (40.0%)
IFN/RBV+BOC or TVR	4 (3.1%)
DAA for compassionate use	3 (2.3%)

Data expressed as median (interquartile range, IQR) and numerical frequencies (percentage). Baseline HCV RNA values in non-cirrhotics were significantly higher compared to those of cirrhotics (T test with Welch's correction; *p value*=0.0017). Interferon, IFN; ribavirin, RBV; boceprevir, BOC; telaprevir, TVR; direct-acting antiviral, DAA.

Clinical monitoring and laboratory assessments

Patients were examined at the Sant'Orsola-Malpighi Hospital Outpatient Unit before starting treatment, on a weekly basis during the first month of therapy and monthly up to 24 weeks post-therapy by means of blood chemistry and liver assessment using the model for end stage liver disease (MELD) and liver fibrosis. In the absence of the fibroscan test, the latter was inferred by calculating Aspartate Aminotransferase (AST) to platelet ratio index (APRI). HCV RNA was quantitated using COBAS TaqMan HCV Quantitative Test version 2.0 (Roche Molecular Diagnostics) with a lower limit of quantitation of 15 IU/mL and genotyped at baseline with VERSANT HCV Genotype 2.0 Assay LiPA (Siemens Healthineers). Both analyses were performed on whole blood samples following automatic RNA extraction.

Resistance-associated substitutions (RASs) were determined by Sanger sequencing of NS3, NS5A, and NS5B regions. NS3 and NS5A were amplified with a nested PCR and NS5B with two overlapping PCRs. Amplifications and sequencing were carried out with Deep Check RT-PCR and Sequencing Assay (Advanced Biological Laboratories) according to the manufacturer's instructions.

Statistical analyses

Data were expressed as median (inter-quartile range, IQR) or mean (standard deviation) for continuous variables and numerical and/or percentage proportions for categorical variables. T Student's test or T test with Welch's correction, Mann-Whitney test and ANOVA were used to calculate statistical differences. The SVR predictive model was obtained from univariate and multivariate analyses. SPSS version 21.0 and GraphPad Prism 5.0 software were used for statistical analyses. A p value less than 0.05 was considered statistically significate.

RESULTS

Description of the study cohort

Clinical and virological features of study patients are shown in *Table 1*. The male gender was predominant (68.1%), and most patients were close to or above 60 years of age and infected by genotype 1b (47%). As regards comorbidities, cardiopulmonary diseases affected nearly half the patients (48.8%) and alcohol abuse was associated with 54.3% of multifactorial liver disease cases. Cirrhosis and severe fibrosis were reported in 48.1% and 16.5% of patients, respectively. The most common liver complications were esophageal varices and HCC, which were present in 25% and 21% of total patients, respectively, and in 64.3% and 75.4% of cirrhotic (C) patients.

Baseline blood chemistry values of the study population comparing cirrhotic (C) to non-cirrhotic (NC) patients are reported in the Supplementary Material (*Table 1*).

Sustained virological response: analyses of incidence and influence on blood chemistry parameters

SVR 24 weeks post-therapy (SVR24) was 90.4% (235/260) (*Figure 1*). Success of therapy ranged from 83.2 to 93.6% in the so-called hard-to-treat categories: 93.6% in patients aged >60, 93.5% in OLT recipients, 85% in previously virological failure (VF) patients, and 83.2% in C-patients. SVR24 was also low in patients with platelet values below reference limits (85.9%), MELD score >10 (83.6%) and APRI score >1 (86.3%).

SVR24 also varied depending on DAA regimen: 75% SOF+RBV, 80.4% SMV±RBV, 94.3% DCV±RBV, 98.7% LDV±RBV and 100% OBV/PTV/r+RBV or OBV/PTV/r+DSV±RBV (*Figure 2*). Evaluation of SVR24 according to DAA treatment and HCV genotype showed good performances of all treatments for genotypes 1a and 1b except SMV±RBV. Drug response of genotypes 2, 3 and 4 varied depending on treatment. Full data are provided in *Figure 1* of the Supplementary Material.

Changes of drugs and regimens from SOF+RBV to OBV/PTV/r+RBV or OBV/PTV/r+DSV±RBV that occurred from 2015 to 2016 increased SVR24 84.4% to 99.0% (Mann-Whitney Test, p value <0.0001). SVR24 was the highest with genotype 1b (95.9%) and decreased progressively with genotype 2 (94.3%), 1a (88.4%), 4 (83.3%), and 3 (75%).

Blood chemical parameters of all SVR24 patients were investigated at the start of therapy (T0), at end-of-treatment (EOT), at 12 (SVR12) and 24 (SVR24) months after therapy. Full data are shown in Table 2 of the Supplementary Material. As expected, AST and ALT decreased during therapy in all patients including C- and NC-groups: T0 AST=52 (32-81) UI/mL and ALT=52.5 (34-83.2) UI/mL vs EOT AST=21 (18-28) UI/mL and ALT=17 (13-24.2). No changes were observed thereafter. A moderate improvement was also observed for platelet values that were 144 (95-196) x10^3/µL at baseline and reached 162 (106-211.5) x10^3/µL at EOT. As opposed to liver enzymes, platelet increase did not reach statistical significance. APRI score dropped significantly from T0 and EOT to stabilize thereafter. MELD score was consistently below <10 and other parameters showed no significant changes throughout the study.

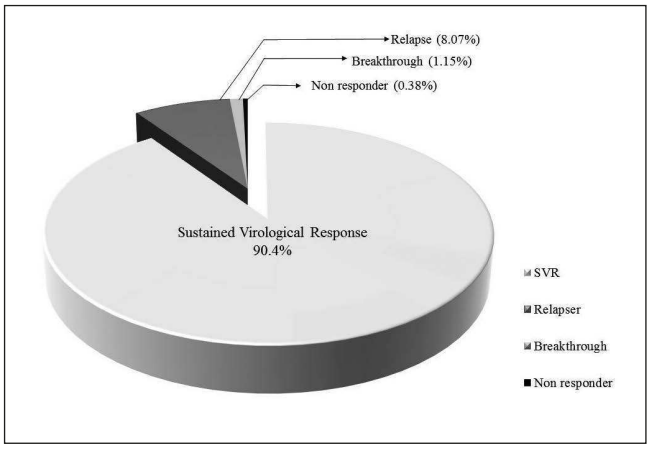

Figure 1 - *Overall virological response rates. Sustained virological response, SVR.*

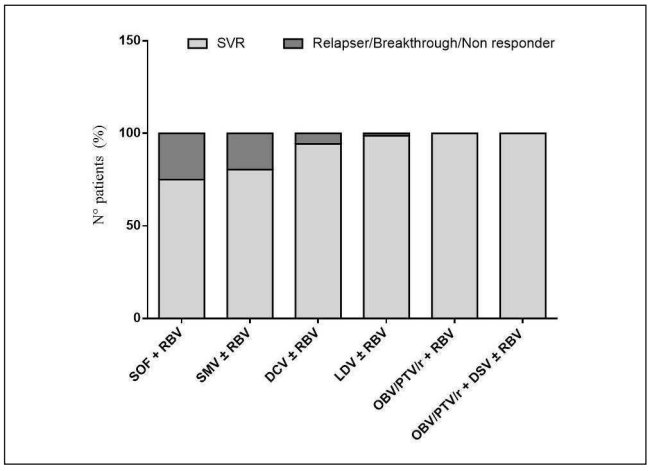

Figure 2 - *Analysis of DAA regimens and virological response. Sofosbuvir (SOF)+ribavirin (RBV); SOF+simeprevir (SMV)±RBV; SOF+daclatasvir (DCV)±RBV, SOF+ledipasvir (LDV)±RBV, ombitasvir/paritaprevir/ritonavir (OBV/PTV/r)+RBV and OBV/PTV/r+dasabuvir (DSV)±RBV.*

Analysis of virological failure

As shown in *Figure 1*, 9.6% of patients experienced VF. In these patients, HCV reactivated at EOT (defined as relapse) in 21 patients (8.07%), during treatment (breakthrough) in 3 patients (1.15%), or did not stop replication

at all (non-response) in 1 patient (0.38%). Demographic, clinical and virological profiles of each VF patient are reported in Table 3 of the Supplementary Material. In the attempt to identify a pattern or a peculiar feature correlated to response or to failure of treatment, age, body mass index, viral load, comorbidities, and chemical parameters at T0 were compared between VF and SVR patients (*Table 2*). VF patients showed more severe liver disease with respect to the SVR. These included cirrhosis (84% of VF patients vs 44.2% of SVR patients), liver complications (e.g., ascites, encephalopathy, portal hypertension, esophageal varices) (72% vs 45.1%), and HCC (44%, vs 19.1%). In contrast, there were no differences as regards viral load and most hemato-chemical parameters, except platelets (lower in VF) and bilirubin (higher in VF) and resulting APRI and MELD. Unsurprisingly, patients who did not respond to previous treatments were over-represented in the VF group (60% vs 36.1%) (*Table 2*).

Only the HCV genome of twelve VF patients was sequenced to search for RAS. These were found in five VF subjects but only two of them bore two NS3 regions RAS (D168V and F43C) for the administered drug (SMV). Unfortunately, no samples at baseline or earlier were available for the two patients to check whether these RAS were present before therapy.

Side effects

During DAA therapy, 41.1% of patients manifested one or more of the following side effects: asthenia (61%), headache (14.9%), itching (12.1%), insomnia (9.3), hyperbilirubinemia (8.4%), anemia (7.4%), nausea/vomiting (6.5%), and diarrhea (5.6%) (Table 4 of the Supplementary Material). Adverse effects did not require intensive care or hospital admission of patients.

To evaluate whether such noxious effects were due to RBV, which was present in four treatments, patients co-treated with RBV (N=14, +RBV groups) were compared to patients who received no RBV (N=146, -RBV group). No significant variations were observed between the two groups and only 16 out of the 107 +RBV patients who reported relevant side effects benefited from RBV dose reduction (13/16) or suspension (3/16).

Is alpha-fetoprotein a marker of virological response?

Since alpha-fetoprotein (AFP) is considered a cancer marker for HCC and other non-liver tumors (Terentiev AA and Moldogazieva NT, 2013), this analysis was restricted to patients with no HCC and for whom the data were available throughout the study. Regardless of the drug treatment, AFP decreased in all 88 study patients (Table 5 of the Supplementary Material, p-value=0.0012). AFP halved by the end of treatment in all C SVR patients (*Figure 3*), while a modest reduction was observed among the VF or NC SVR patients (Table 5 of the Supplementary Material). As a whole, the higher the AFP value at baseline, the faster its reduction.

Hepatocellular carcinoma and DAA therapy

HCC occurred in 2 (2/204, 0.98%) patients, both achieving SVR24 and one affected by cirrhosis. HCC recurrence was observed in 8 HCV patients with HCC during treatment (8/56, 14.28%) and after a median value of three months

Table 2 - *Comparative analyses between patients experiencing virological failure or sustained virological response (SVR).*

	Virological failure	*Sustained virological response*	*p value*
Demographic data			
Age (yr)	56 (51.5-63)	60 (53-72)	0.0223
BMI	27 (21.2-35.5)	25.5 (23.4-28.7)	0.2516
Clinical aspects			
Cirrhosis	84% (21/25)	44.2% (104/235)	0.0002*
Liver complications	72% (18/25)	45.1% (99/235)	0.0002*
MELD	11 (9.5-13)	8 (7-10)	0.0253*
APRI	2.29 (0.93-4.09)	1.03 (0.46-1.95)	0.0370*
HCC	44% (11/25)	19.1% (45/235)	0.0068*
Virology			
HCV RNA (UI/mL)	1,069,083 (398,301-1,969,892)	1,253,438 (319,706-3,053,024)	0.4951
Therapy			
Not naïve	60% (15/25)	36.1% (85/235)	0.0291*
Baseline blood chemistry			
Hemoglobin (g/dL)	13.35 (11.2-14.6)	13-7 (12.6-14.9)	0.2191
Platelets ($x10^3/\mu L$)	78 (64-125.5)	144 (95-196)	<0.0001*
PT (INR)	1.27 (1.15-1.33)	1.07 (1-1.18)	0.0476*
Creatinine (mg/dL)	0.69 (0.66-0.88)	0.89 (0.71-1.1)	0.2101
Sodium (mmol/L)	140 (137-141)	140 (138-142)	0.3233
Albumin (g/dL)	3.5 (3.4-3.8)	3.9 (3.3-4.3)	0.0675
Bilirubin (mg/dL)	1.61 (0.91-2.19)	0.79 (0.56-1.13)	0.0161*
AST (U/L)	66 (35.5-108.5)	52 (32-81)	0.4965
ALT (U/L)	54 (31-93)	52.5 (34-83.2)	0.6073
ALP (U/L)	99 (88-119)	98 (74-142)	0.5080
AFP (ng/mL)	13 (5-20)	7 (3-12)	0.8030

Data reported as median (interquartile range, IQR) and numerical frequency (percentage). Body mass index, BMI; model for end-stage liver disease, MELD; AST; aspartate aminotransferase, AST to platelet ratio index, APRI; prothrombin time, PT; alanine aminotransferase, ALT; alkaline phosphatase, ALP; alpha-fetoprotein, AFP.

Table 3 - *Analyses of predictive models of sustained virological response.*

		Univariate		*Multivariate*	
	SVR24 n/N (%)	*ODDS RATIO (95%CI)*	*p value*	*ODDS RATIO (95%CI)*	*p value*
Demographic data					
Age >60 yr	117/125 (93.6)	2.107 (0.875-5.071)	0.397		NA
Sex M	158/177 (89.3)	0.648 (0.248-1.688)	0.543		NA
BMI ≥30	24/29 (82.7)	0.455 (0.156-1.322)	0.389		NA
Comorbidities					
Cardiopulmonary	118/127 (95.9)	1.793 (0.761-4.219)	0.326		NA
Diabetes	42/49 (85.7)	0.559 (0.219-1.424)	0.427		NA
Renal insufficiency	18/20 (90.0)	0.953 (0.208-4.373)	0.989		NA
Liver					
Cirrhosis	104/125 (83.2)	0.151 (0.050-0.454)	0.472		0.130
A, VE, PH	99/120 (82.5)	0.138 (0.461-0.416)	0.867		0.924
HCC	45/56 (80.3)	0.301 (0.128-0.708)	0.156		0.079
OLT	44/46 (95.6)	2.649 (0.602-11.656)	0.357		NA
Extraliver					
Cryoglobulinemia	21/23 (91.3)	1.128 (0.248-5.123)	0.885		NA
Baseline virology/blood chemical data					
Log HCV RNA ≥6	184/204 (90.2)	0.902 (0.322-2.521)	0.993		NA
Platelets (x10³/μL) <100	69/87 (79.3)	0.161 (0.064-0.404)	0.650		0.792
Bilirubin (mg/dL) >0.8	109/129 (84.5)	0.216 (0.078-0.595)	0.433		0.643
MELD ≥10	73/92 (79.3)	0.142 (0.054-0.371)	0.008*	0.125 (0.031-0.234)	0.011*
APRI ≥1	120/139 (86.3)	0.329 (0.127-0.854)	0.263		0.341
Previous VF	85/100 (85.0)	0.377 (0.162-0.877)	0.041		0.072
IV Week therapy					
HCV RNA (UI/mL) <15	200/220 (90.9)	0.700 (0.246-1.987)	0.729		NA
Platelets (x10³/μL) >100	179/186 (96.2)	0.121 (0.048-0.306)	0.700		0.661
AST (U/L) ≤30	185/200 (92.5)	0.405 (0.171-0.957)	0.198		0.265
ALT (U/L) ≤30	185/206 (89.8)	1.418 (0.465-4.322)	0.686		NA
Bilirubin (mg/dL) ≤1	136/144 (94.4)	0.416 (0.176-0.981)	0.234		0.176
APRI ≤0.8	187/195 (95.8)	0.120 (0.049-0.296)	0.166		0.870
VIII Week therapy					
HCV RNA (UI/mL) <15	232/256 (90.6)	0.310 (0.031-3.101)	0.457		NA
Platelets (x10³/μL) >100	184/193 (95.3)	0.155 (0.065-0.373)	0.668		0.518
AST (U/L) ≤20	104/110 (94.5)	0.397 (0.153-1.031)	0.079		NA
ALT (U/L) ≤20	147/154 (95.4)	0.232 (0.093-0.579)	0.016*	0.463 (0.076-0.791)	0.014*
Bilirubin (mg/dL) ≤0.8	108/116 (93.1)	0.553 (0.229-1.332)	0.338		NA
APRI ≤0.6	172/180 (95.5)	0.172 (0.070-0.419)	0.651		0.351

Abbreviations: sustained virological response, SVR; body mass index, BMI; ascites, A; esophageal varices, EV; portal hypertension, PH; model for end-stage liver disease, MELD; AST platelet ratio index, APRI; virological failure, VF; aspartate aminotransferase, AST; alanine aminotransferase, ALT.

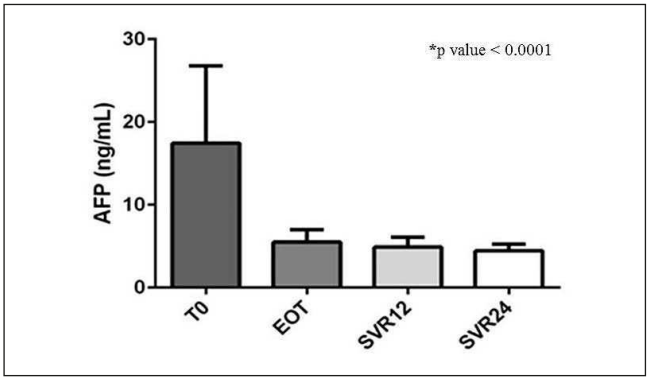

Figure 3 - *AFP levels in SVR cirrhotic patients. ANOVA was performed to compare alpha-fetoprotein (AFP) values at T0, end of therapy (EOT), SVR12 e SVR24 of cirrhotic patients with successful DAA response.*

from the start of therapy (1.25-11.25); of these, 6 patients (75%) reached SVR. To reduce HCC, all patients underwent local-regional treatments (e.g., percutaneous ethanol injection, radiofrequency ablation and transcatheter arterial chemoembolization) and only 4 patients underwent resection.

Success of antiviral treatment: looking for predictors

In an attempt to find (early) predictors of SVR, demography, comorbidities, liver and extra-liver complications, virological and blood chemistry parameters at baseline, at 4 and 8 weeks of therapy were thoroughly analyzed (*Table 3*). Among all parameters, T0 MELD score ≥10 and ALT >20 after 8 weeks of DAA treatment were associated with a high risk of VF. This result led us to propose MELD <10 at T0 and ALT ≤20 after 8 weeks of therapy as positive predictors of DAA response.

DISCUSSION

This monocentric, retrospective, study evaluated the efficacy, safety and predictors of virological response to various anti-HCV DAA treatments. Compared to previous studies, this was conducted in a heterogenous cohort of patients with chronic HCV infection and characterized by mild to severe stages of liver disease, with prior VF, transplanted or waiting for OLT. The novelty of this survey is, therefore, that the HCV drugs were evaluated in a real clinical setting where each patient had his/her own clinical story and needed specific treatment. Overall, SVR was >90%, a rate confirming the outstanding potency of DAAs but slightly lower compared to two similar retrospective studies carried out in Germany (93.7%) (Werner CR *et al*., 2016) and Brazil (93.5%) (Cheinquer H *et al*., 2017). This discrepancy could be due to the study population, in which 48.1% were C-patients compared to 34% in the German study (Werner CR *et al*., 2016). Furthermore, SOF that, together with RBV was the first IFN-free DAA treatment, was administered to only 4.3% of Brazilian patients compared to 18.5% in our study (Cheinquer H *et al*., 2017). Excluding difficult-to-treat patients, SVR24 increased to 97% and fell to 83.2% and 85% including C and previous VF patients. Similarly, SVR24 was lower considering parameters known to affect therapy performance, such as low platelet values (85.9%), MELD >10 (83.6%) and APRI score >1 (86.3%) (Myers RP *et al*., 2015). Finally, OLT patients, including hard-to-treat, showed 93.5% SVR, in agreement with a previous study performed in recipients of organ transplants (Saxena V *et al*., 2017). Finally, and again in line with previous reports, patients >60 years responded better (93.6% SVR) than patients ≤60 years (87.4%), and genotype 3 was the most difficult to treat (Su F *et al*., 2017; Fathi H *et al*., 2017).

As regards SVR24 relative to each DAA regimen, SOF+RBV and SMV±RBV were the least effective (SVR ~80%) as opposed to DCV±RBV, LDV±RBV, OBV/PTV/r+RBV and OBV/PTV/r+DSV±RBV, which reached 94% SVR. In published clinical trials, SOF+RBV treatment achieved 50% to 100% SVR depending on length of therapy (12, 16, or 24 weeks), HCV genotype, severity of liver disease and previous VFs (Brai A *et al*., 2016; Crouchet E *et al*., 2018; Zeuzem S *et al*., 2014; Isakov V *et al*., 2016). In reference studies OPTIMIST-1 and -2, C and NC-patients infected by HCV genotype-1 and treated with SMV±RBV reached SVR12 96.8% and 83.5%, respectively (Kwo P *et al*., 2016; Lawitz E *et al*., 2016). In contrast, SVR of the same treatment against HCV genotype 4 ranged 96.0% to 65.4% depending on severe liver disease, cirrhosis and previous VF (Moreno C *et al*., 2015; Lawitz E *et al*., 2013). High VF rates were found with SOF+RBV and SMV±RBV regimens, in line with previous studies (Werner CR *et al*., 2016). A phase II study to test DCV±RBV, carried out in 2013-2014, showed 98%, 92%, and 89% SVR for genotype 1, 2, and 3, respectively (Sulkowski MS *et al*., 2014). In our study, SVR was 100% for genotype 1a, 1b and 2, and 95.2% for genotype 3. In the current study, this regimen was also tested against genotype 4, for which a breakthrough case was observed. Another effective DAA regimen was LDV±RBV, which yielded a 98.7% SVR rate similar to phase III clinical trials (a. Afdhal N *et al*., 2014; b. Afdhal N *et al*., 2014) or retrospective studies in heterogeneous cohorts (Werner CR *et al*., 2016; Cheinquer H *et al*., 2017; Afdhal N *et al*., 2017). Finally, OBV/PTV/r+RBV and OBV/PTV/r+DSV±RBV, reached 100% SVR in patients with cirrhosis or previous VF. Such a remarkable performance was also observed in previous phase II or III clinical studies carried out in homogeneous or heterogeneous patient populations in which SVR ranged from 91.8 to 100% (Werner CR *et al*., 2016; Cheinquer H *et al*., 2017; Ferenci P *et al*., 2014; Poordad F *et al*., 2014). Overall, this and reported studies demonstrate that DAA therapy is highly efficient in all clinical settings and also performs well in HCV-infected patients with heterogeneous and advanced stages of liver disease. There are still some hard-to-treat categories who do not respond to current DAAs. Elbasvir/grazoprevir for genotypes -1 and -4 and pan-genotypic SOF/velpatasvir and glecaprevir/pibrentasvir treatments, recently approved by the European Medicines Agency, could resolve this issue (Asselah T *et al*., 2017; Jacobson IM *et al*., 2017; Kumada H *et al*., 2018).

During treatment, some hematological and chemical parameters in C- and NC-patients were examined. Besides a marked decrease of liver necrosis markers, APRI score also significantly diminished, indicating a substantial regression of liver damage. In contrast with previous studies, no significant increase of bilirubin levels in +RBV patients was observed (Juanbeltz R *et al*., 2017). Other hematochemical parameters were within the normal range, confirming the optimal safety profile of DAAs. This conclusion is also supported by mild side effects reported in all studies, including ours (Werner CR *et al*., 2016).

AFP is a marker of HCC and non-liver cancer but its reliability is questionable in HCV patients since it also increases during infection, perhaps as consequence of chronic liver inflammation (Di Bisceglie AM *et al*., 2005). AFP fluctuations thoroughly examined in IFN-based therapy and in patients affected by HCV or hepatitis B virus infections were largely ignored in IFN-free therapy studies (Nguyen K *et al*., 2017). It was known that AFP diminishes during IFN treatment only if patients have compensated cirrhosis (Nguyen K *et al*., 2017). In this study, AFP reduction proved significant not only in SVR patients with compensated cirrhosis but also with decompensated cirrhosis. It can be inferred, therefore, that normalization of AFP in HCV patients is a consequence of reduced liver inflammation under DAA treatment, thus making AFP a valuable cancer marker for HCC screening in these patients as well. Concerning the hypothesized role of DAA therapy in promoting insurgence or recurrence of HCC, our study does not support this theory since the rate of HCC recurrence and occurrence was lower than in several retrospective and prospective studies (Guarino M *et al*., 2018). Further studies in large cohorts of patients are required to address this issue.

Unfortunately, no blood samples collected before therapy were available for evaluation of possible correlations between RAS and response to therapy. This analysis was thus performed only in 12 patients who did not respond to therapy. RAS within NS3-NS5 regions correlated to VFs were detected in 16.6%, a rate lower than in a recent multicenter study in which NS3 and NS5A RAS were found in 49.5% of VF patients, much less in NS5B (Di Maio VC *et al*., 2017).

The quest for pathological, biochemical, and virological markers predicting the response to therapy has been pursued since the dawn of HCV therapy. So far, the most reliable positive predictive value is serum HCV RNA level

at the 4th week of therapy (Johnson K *et al*., 2017). Our retrospective analyses found MELD score <10 pre-therapy and a decrease in ALT values during therapy as possible factors predicting SVR. These findings were also confirmed by other studies (Werner CR *et al*., 2016; Khan ST *et al*., 2017). Khan and collaborators correlated a value of 40 U/L and at the 12th week with success of therapy (Khan ST *et al*., 2017). Should our result be confirmed, the novelty compared to previous findings is an earlier inference of efficacy and, above all, at a time when adjustment of therapeutic strategy is still possible. Further, it has been reported that ALT and HCV RNA follow independent kinetic and transaminase decrease even when HCV RNA is undetectable (Cento V *et al*., 2017). This finding suggests that anti-HCV therapy operates on two possible sequential levels: the first is halting of viral replication, monitored by HCV RNA plasma levels; the second, either direct or resulting from incapacity of the virus to replicate, is reduction of liver damage, inferred from ALT levels.

In conclusion, this study demonstrates that DAAs are safe and effective in patients with various types and stages of liver disease, providing a therapeutic opportunity also in hard-to-treat conditions, timely adjustment of therapy being achievable by monitoring rapidity of ALT decrease, and with MELD score <10 pre-therapy being a possible predictor of SVR.

However, the therapeutic regimens under investigation in this study are now being replaced by new combinations of DAAs (Elbasvir/grazoprevir, SOF/velpatasvir and glecaprevir/pibrentasvir). It will be of considerable clinical interest to analyze the power of low values of MELD and ALT reduction with new DAAs to confirm their validity and reliability as predictors of SVR.

Thanks to new DAAs, the eradication of HCV infection is likely an achievable goal. The definition of reliable and handy early predictors of SVR would permit easy gauging and refinements of drug therapy to avoid VF, emergence of resistant strains, and worsening of the disease.

Acknowledgments

We thank the FAS Salute 2014 Regione Toscana for providing partial support to the study.

Conflicts of Interest

The authors declare no financial or other conflicts of interest that might be construed to influence the contents of the manuscript, including the results or interpretation of publication. This study was supported in part by grants of FAS Salute 2014 Regione Toscana "UNAVIR: Malattie virali rare: una strategia innovativa per combatterle con un unico agente antivirale".

References

Afdhal N. (a), Reddy K.R., Nelson D.R., Lawitz E., Gordon S.C., et al. (2014). Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med.* **370**, 1483-1493.

Afdhal N. (b), Zeuzem S., Kwo P., Chojkier M., Gitlin N., et al. (2014). Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med.* **370**, 1889-1898.

Afdhal N., Zeuzem S., Kwo P., Chojkier M., Gitlin N., et al. (2017). Safety and efficacy of sofosbuvir-based direct-acting antiviral regimens for hepatitis C virus genotypes 1-4 and 6 in Myanmar: Real-world experience. *J Viral Hepat.* **24**, 927-935.

Asselah T., Bourgeois S., Pianko S., Zeuzem S., Sulkowski M., et al. (2017). Sofosbuvir/Velpatasvir in Patients With Hepatitis C Virus Genotypes 1-6 and Compensated Cirrhosis or Advanced Fibrosis. *Liver Int.* **38**, 443-450.

Bacon B.R., Gordon S.C., Lawitz E., Marcellin P., Vierling J..M, et al. (2011). Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med.* **364**, 1207-1217.

Banerjee D., Reddy K.R. (2016). Review article: safety and tolerability of direct-acting anti-viral agents in the new era of hepatitis C therapy. *Aliment Pharmacol Ther.* **43**, 674-696.

Brai A., Fazi R., Tintori C., Zamperini C., Bugli F., et al. (2016). Human DDX3 protein is a valuable target to develop broad spectrum antiviral agents. *Proc Natl Acad Sci USA.* **113**, 5388-5393.

Cento V., Nguyen T.H.T., Di Carlo D., Biliotti E., Gianserra L., et al. (2017). Improvement of ALT decay kinetics by all-oral HCV treatment: Role of NS5A inhibitors and differences with IFN-based regimens. *PLoS One.* **12**, e0177352.

Cheinquer H., Sette-Jr H., Wolff F.H., de Araujo A., Coelho-Borges S., et al. (2017). Treatment of Chronic HCV Infection with the New Direct Acting Antivirals (DAA): First Report of a Real World Experience in Southern Brazil. *Ann Hepatol.* **16**, 727-733.

Crouchet E., Wrensch F., Schuster C., Zeisel M.B., Baumert T.F. (2018). Host-targeting therapies for hepatitis C virus infection: current developments and future applications. *Therap Adv Gastroenterol.* **11**, 1756284818759483.

Di Bisceglie A.M., Sterling R.K., Chung R.T., Everhart J.E., Dienstag J.L., et al. (2005). Serum alpha-fetoprotein levels in patients with advanced hepatitis C results from the HALT- C Trial. *J Hepatol.* **43**, 434-441.

Di Maio V.C., Cento V., Lenci I., Aragri M., Rossi P., et al. (2017). Multiclass HCV resistance to direct-acting antiviral failure in real-life patients advocates for tailored second-line therapies. *Liver Int.* **37**, 514-528.

European Association for Study of Liver. (2015). EASL Recommendations on Treatment of Hepatitis C 2015. *J Hepatol.* **63**, 199-236.

European Association for the Study of the Liver. EASL Recommendations on Treatment of Hepatitis C 2016. (2017). *J Hepatol.* **66**, 153-194.

Fathi H., Clark A., Hill N.R., Dusheiko G. (2017). Effectiveness of current and future regimens for treating genotype 3 hepatitis C virus infection: a large-scale systematic review. *BMC Infect Dis.* **17**, 722.

Ferenci P., Bernstein D., Lalezari J., Cohen D., Luo Y., et al. (2014). ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. *N Engl J Med.* **370**, 1983-1992.

Global Hepatitis Report. (2017). World Health Organization.

Guarino M., Sessa A., Cossiga V., Morando F., Caporaso N., et al. (2018). Direct-acting antivirals and hepatocellular carcinoma in chronic hepatitis C: A few lights and many shadows. *World J Gastroenterol.* **24**, 2582-2595.

Isakov V., Zhdanov K., Kersey K., Svarovskaia E., Massetto B., et al. (2016). Efficacy of sofosbuvir plus ribavirin in treatment-naive patients with genotype-1 and -3 HCV infection: results from a Russian Phase IIIb study. *Antivir Ther.* **21**, 671-678.

Jacobson I.M., Lawitz E., Kwo P.Y., Hézode C., Peng C.Y., et al. (2017). Safety and Efficacy of Elbasvir/Grazoprevir in Patients With Hepatitis C Virus Infection and Compensated Cirrhosis: An Integrated Analysis. *Gastroenterology.* **152**, 1372-1382.e2.

Johnson K., Green P.K., Ioannou G.N. (2017). Implications of HCV RNA level at week 4 of direct antiviral treatments for hepatitis C. *J Viral Hepat.* **24**, 966-975.

Juanbeltz R., Goñi Esarte S., Úriz-Otano J.I., Martínez Echeverría A., Elizalde I., et al. (2017). Safety of oral direct acting antiviral regimens for chronic hepatitis C in real life conditions. *Postgrad Med.* **129**, 476-483.

Khan S.T., McGuinty M., Corsi D.J., Cooper C.L. (2017). Liver enzyme normalization predicts success of Hepatitis C oral direct-acting antiviral treatment. *Clin Invest Med.* **40**, E73-E80.

Kumada H., Watanabe T., Suzuki F., Ikeda K., Sato K., et al. (2018). Efficacy and safety of glecaprevir/pibrentasvir in HCV-infected Japanese patients with prior DAA experience, severe renal impairment, or genotype 3 infection. *J Gastroenterol.* **53**, 566-575.

Kwo P., Gitlin N., Nahass R., Bernstein D., Etzkorn K., et al. (2016). Simeprevir plus sofosbuvir (12 and 8 weeks) in hepatitis C virus genotype 1-infected patients without cirrhosis: OPTIMIST-1, a phase 3, randomized study. *Hepatology.* **64**, 370-380.

Lawitz E., Mangia A., Wyles D., Rodriguez-Torres M., Hassanein T., et al. (2013). Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med.* **368**, 1878-1887.

Lawitz E., Matusow G., DeJesus E., Yoshida E.M., Felizarta F., et al. (2016). Simeprevir plus sofosbuvir in patients with chronic hepatitis C virus genotype 1 infection and cirrhosis: a phase 3 study (OPTIMIST-2). *Hepatology.* **64**, 360-369.

Lindsay K.L. (1997). Therapy of hepatitis C: overview. *Hepatology.* **26**, 71S-77S.

Moreno C., Hezode C., Marcellin P., Bourgeois S., Francque S., et al. (2015). Efficacy and safety of simeprevir with PegIFN/ribavirin in naïve or experienced patients infected with chronic HCV genotype 4. *J Hepatol.* **62**, 1047-1055.

Myers R.P., Shah H., Burak K.W., Cooper C., Feld J.J. (2015). An update on the management of chronic hepatitis C: 2015 Consensus guidelines

from the Canadian Association for the Study of the Liver. *Can J Gastroenterol Hepatol.* **29**, 19-34.
Nguyen K., Jimenez M., Moghadam N., Wu C., Farid A., et al. (2017). Decrease of Alpha-fetoprotein in Patients with Cirrhosis Treated with Direct-acting Antivirals. *J Clin Transl Hepatol.* **5**, 43-49.
Polaris Observatory HCV Collaborators. (2017). Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *Lancet Gastroenterol Hepatol.* **2**, 161-176.
Poordad F., Hezode C., Trinh R., Kowdley K.V., Zeuzem S., et al. (2014). ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. *N Engl J Med.* **370**, 1973-1982.
Saxena V., Khungar V., Verna E.C., Levitsky J., Brown R.S. Jr, et al. (2017). Safety and Efficacy of Current DAA Regimens in Kidney and Liver Transplant Recipients with Hepatitis C: Results from the HCV-TARGET Study. *Hepatology.* **66**, 1090-1101.
Su F., Beste L.A., Green PK., Berry K., Ioannou G.N. (2017). Direct-acting antivirals are effective for chronic hepatitis C treatment in elderly patients: a real-world study of 17 487 patients. *Eur J Gastroenterol Hepatol.* **29**, 686-693.
Sulkowski M.S., Gardiner D.F., Rodriguez-Torres M., Reddy KR, Hassanein T., et al. (2014). Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. *N Engl J Med.* **370**, 211-221.
Terentiev A.A., Moldogazieva N.T. (2013). Alpha-fetoprotein: a renaissance. *Tumour Biol.* **34**, 2075-2091.
Zeuzem S., Andreone P., Pol S., Lawitz E., Diago M., et al. (2011). Telaprevir for retreatment of HCV infection. *N Engl J Med.* **364**, 2417-2428.
Zeuzem S., Dusheiko G.M., Salupere R., Mangia A., Flisiak R., et al. (2014). Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med.* **370**, 1993-2001.
Werner C.R., Schwarz J.M., Egetemeyr D.P., Beck R., Malek N.P., et al. (2016). Second-generation direct-acting-antiviral hepatitis C virus treatment: Efficacy, safety, and predictors of SVR12. *World J Gastroenterol.* **22**, 8050-8059.