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ORIGINAL PAPERS

Influence of sustained viral response on the regression of fibrosis and portal hypertension in cirrhotic HCV patients treated with antiviral triple therapy

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ABSTRACT

Background and aims: The regression of liver fibrosis and portal hypertension (PH) and their influence on the natural history of compensated hepatitis C virus (HCV)-related cirrhosis has not been studied previously. Our objective was to evaluate the influence of sustained virologic response (SVR) on the portal pressure gradient (HVPG) and non-invasive parameters of PH and prognostic factors of response.

Methods: Sixteen patients with compensated HCV genotype 1-related cirrhosis with PH (HVPG > 6 mmHg) without beta-blocker therapy were considered as candidates for PEGα2a + RBV + BOC (48 weeks; lead-in and accepted stopping rules). A hemodynamic study and Fibroscan® were performed at baseline, at eight weeks and, in the case of SVR, 24 weeks after treatment. In each hemodynamic study, serum samples were analyzed for inflammatory biomarkers associated with PH.

Results: In eight cases, SVR was obtained; five patients relapsed, and treatment was stopped early for non-response to lead in (one case) and a decrease of < 3 log at week 8 (two patients). Compared to baseline, there was a significant decrease in HVPG and Fibroscan® at weeks 8 and 72 (10.31 ± 4.3 *vs* 9.4 ± 5.04 *vs* 6.1 ± 3.61 mmHg, p < 0.0001 and 21.3 ± 14.5 *vs* 16.2 ± 9.5 *vs* 6.4 ± 4.5 kPa, p < 0.0001, respectively). The average HVPG decrease in SVR was 40.8 ± 17.53%, achieving an HVPG < 6 mmHg in five patients (62.5%) and a Fibroscan® < 7.1 kPa in three patients (37.5%).

Conclusions: Complete hemodynamic response (HVPG < 6 mmHg) and fibrosis regression (Fibroscan® < 7.1 kPa) occur in more than half and one-third of patients achieving SVR, respectively, and must be another target in cirrhotic patients with SVR.

Key words: Portal pressure gradient. Portal hypertension. Triple therapy. SVR. Fibroscan®.

INTRODUCTION

The emergence of the first-generation protease inhibitors, Boceprevir (BOC) and Telaprevir associated with peginterferon + ribavirin (PR) allowed for the first time the treatment of cirrhotic patients with clinically significant portal hypertension, with a response rate of approximately 40-50%. However, treatment in patients with advanced liver disease is prolonged, with many side effects and a poor response rate (40-70%). The stopping rules at weeks 4, 8, 12 and 24 are an essential tool to prevent continued treatment in patients with a low probability of response and who are at risk of developing serious adverse effects (1-5). These treatments were the first step in IFN-free regimens, with higher SVR rates and less adverse effects (3).

While in previous studies in non-cirrhotic patients the antiviral target of hepatitis C liver disease is to achieve a sustained virologic response (SVR), normalization of ALT/AST and improvement in the degree of liver fibrosis (6-9), in patients with advanced liver disease and signs of portal hypertension a third objective must be the regression, and even normalization, of the hepatic venous pressure gradient (HVPG), the gold standard of portal pressure measurement (10).

Rincón et al. conducted a controlled study to assess the usefulness of HVPG compared with liver biopsy as the gold standard of a histological response in HCV cirrhotic patients. Twenty patients with HCV genotype 1b-related compensated cirrhosis (HVPG > 5 mmHg) underwent treatment with standard antiviral therapy (interferon and ribavirin) (10). They achieved a mild HVPG decrease of 28.2%. However, the decrease was significantly higher in SVR patients than in partial responders or non-responders (26.2% *vs* 12.7%, respectively). The hemodynamic response was a significantly more dynamic marker of severity, disease progression and response to antiviral treatment than liver biopsy. As in 75% of cases, there was no change in the METAVIR stage on post-treatment biopsy. Thus, they were able to conclude that a decrease of at least 20% in HVPG compared to baseline could be considered as another independent marker of SVR (10). Therefore, HCV treatment was shown to be an excellent anti-fibrotic therapy and was able to improve intrahepatic vascular resistance and portal hypertension. These data confirmed the results obtained by Roberts et al. (8), which

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suggested that in cirrhotic patients, treatment with interferon for 48 weeks caused an average decrease of 20% in HVPG compared to baseline.

However, HVPG measurement is an invasive method and not available in all centers; thus, in recent years, there have been numerous studies attempting to validate the usefulness of non-invasive methods such as elastography (11-20) and serum inflammatory biomarkers (21-24) compared with HVPG.

The objective of this study was to evaluate the usefulness of HVPG, Fibroscan® and serological biomarkers of inflammation as predictors of the response to peginterferon α2a (pegIFN) + ribavirin (RBV) and boceprevir (BOC) (in a 48-week schedule) in patients with HCV genotype 1-related cirrhosis and portal hypertension. The additional objectives were to assess the regression of fibrosis and portal hypertension in association with SVR.

MATERIALS AND METHODS

This study was conducted in the Gastroenterology and Hepatology Department of the Hospital Universitario Marqués de Valdecilla (Santander, Spain) and at the Hospital de Sierrallana (Torrelavega, Spain). The study was approved by the Cantabria Ethics Committee (CEIC).

Patient selection

From October 2012 to August 2013, 20 patients with analytical (platelet count below 100,000/mm^3), elastographic (Fibroscan® > 8 kPa) or hemodynamic criteria (HVPG > 6 mmHg) of HCV genotype 1-related liver cirrhosis and portal hypertension were consecutively recruited. The inclusion criteria were as follows: a) compensated HCV genotype 1-related liver cirrhosis diagnosed by clinical, laboratory, ultrasound or biopsy criteria; b) portal hypertension defined as an HVPG > 6 mmHg; c) a detectable viral load; and d) written informed consent. The exclusion criteria included one or more of the following: a) age < 18 or > 80 years; b) alcoholic, HBV-related, autoimmune, metabolic or cryptogenic liver cirrhosis; c) alcohol consumption; d) contraindications to antiviral treatment; e) thrombosis in the splenoportal axis; f) hepatocarcinoma; g) a Child-Pugh score > 8 points; h) any comorbidity involving a limited life expectancy (< 12 months); i) refusal to participate in the study, and informed consent claim; j) pregnancy or lactation; and k) HIV co-infection.

All patients who were potential candidates for the study and had non-invasive data on HCV-related cirrhosis with mild to severe portal hypertension underwent first a hemodynamic study. Those patients in whom a portal pressure gradient greater than 6 mmHg was found were considered to be potential candidates for study inclusion. Those with an HVPG < 6 mmHg were excluded from the trial.

Study design

All patients who met the inclusion criteria in the absence of any exclusion criteria were included in the study. Treatment was prescribed according to the Summary of the Product Characteristics of each of the anti-HCV drugs. Specific recommendations established by the Spanish Agency of Drugs and Sanitary Products regarding treatment schedules, recommended duration and treatment stopping rules were followed. All included patients were undergoing pegIFN + RBV + BOC treatment for 48 weeks with lead in.

Baseline routine laboratory tests were performed, including hemogram, INR, prothrombin activity, biochemistry (AST, ALT, GGT, FA, bilirubin, albumin, cholesterol, and insulin), and viral load (quantitative PCR, Roche assay UI and logarithmic). In addition, special analytical determinations were performed while the hepatic hemodynamic procedure was carried out using inferior vena cava blood after 30 min of resting in a supine position (renin, aldosterone, IL-6, and inflammatory biomarkers: VCAM 1, IL-1ß and IL-1Rα). Baseline imaging tests (abdominal ultrasound, chest X-ray and hemodynamic study), endoscopic studies (conventional gastroscopy screening for esophageal varices) and Fibroscan® were performed on all patients.

Patients were evaluated at least on a monthly basis in an outpatient clinic or when treatment adjustment was required or adverse side effects were identified. During all visits, a complete analysis with viral load was performed. FibroScan® and hemodynamic study with special analytical tests were repeated at eight weeks and six months post-treatment (in the group of patients without SVR the last hemodynamic study was not performed due to ethical reasons). The objective was to study the value of HVPG, Fibroscan® at baseline and eighth week as prognostic markers of antiviral response, regression of fibrosis and portal hypertension.

Scheme of antiviral treatment

Patients were initially treated with pegIFN (180 microgram subcutaneous dose/week) plus RBV (1,000 mg per day divided into two doses for patients weighing 75 kg or less or 1,200 mg per day divided into two doses for patients exceeding 75 kg) for four weeks (lead in). If a decrease in viral load (VL) > 1 log was achieved, BOC at a dose of 800 mg three times a day was added to the treatment regimen. Treatment with three drugs was completed at week 48. The considered stopping rules were as follows: VL decrease less than 3 log at week 8, VL > 1,000 IU at week 12, detectable VL at week 24 or an increase in VL at any time during treatment.

Hemodynamic study

Hemodynamic studies were performed after an overnight fast. Under local anesthesia, a catheter introducer was placed in the right internal jugular vein using the Seldinger technique and was used to advance, under fluoroscopic guidance, a 7-F balloon-tipped catheter into the right main hepatic vein and a Swan-Ganz catheter into the pulmonary artery. Portal pressure was measured as the HVPG, which is the difference between wedged and free hepatic venous pressure. All intravascular pressure measurements were performed in triplicate using a previously calibrated, highly sensitive transducer, with external zero at the mid-axillary line; a permanent recording of tracings was obtained. The occluded position was confirmed by the absence of reflux after injection of contrast medium. Electrocardiography, arterial pressure, heart rate, and

oxygen saturation were monitored noninvasively throughout the study with an automatic monitor.

Fibroscan®

Elastography studies were carried out after an overnight fast the same day as the hemodynamic study by the same senior technician, with an experience of more than 2,000 procedures. All determinations were adjusted to accepted quality criteria (IQR/median < 30%, success rate > 60%; EASL - Clinical Practice Guidelines: Non-invasive tests for the evaluation of liver disease severity and prognosis, 2015).

Serum sample procedures

After completing hemodynamic measurements, blood samples for renin, aldosterone and inflammatory biomarkers were obtained from the inferior vena cava. Inflammatory biomarker samples were centrifuged and preserved at -70 °C in the IDIVAL laboratory. At the end of the study, the blood samples were analyzed for novel inflammatory biomarkers. We analyzed the following markers by ELISA: IL-1Beta, IL-1R-Alpha, VCAM 1 and IL-6 according to the manufacturers' protocols (Human IL-1Bβ ELISA Kit, RayBio ELH-1L1β, correlation coefficient 0.971; Human IL-1Rα Platinum ELISA, eBioscience, Ref.: BMS2080/BMS2080TEN correlation coefficient 1.00; Human VCAM-1 ELISA Kit, Fabricant: Booster, Ref.: EK0537, correlation coefficient 1.00; IL-6, Human IL-6, Cusabio, Ref.: CSB-E04638h, correlation coefficient 0.992).

Safety assessments

Adverse effects (AEs) data were collected for all subjects during the treatment period and during follow-up, until the 24th week after treatment. These data included clinical and laboratory grade 3 or 4 AEs, serious adverse events (SAEs), and special interest events, such as grade 2 or higher anemia events, infections, hepatic decompensation and other cytopenias.

The management of the anemia events was first based on reducing the dose of RBV, followed by the administration of hematopoietic growth factors (erythropoietin) and in the case of non-response or severe anemia, by blood transfusion. The use of growth factors needed to manage other cytopenias, such as neutropenia or thrombocytopenia, was also recorded.

The following factors were assessed for a potential relationship with safety events: baseline MELD score, baseline HVPG value, Fibroscan®, esophageal varices (presence/absence), platelet count, serum albumin (< 3.5 mg/dl), bilirubin level, and baseline hemoglobin level.

Statistical analysis

Statistical analysis was performed according to an intention-to-treat strategy. Categorical variables, which were reported as frequencies, were compared using the Fisher's exact test. Continuous variables, which were reported as the median value and standard deviation, were compared using the unpaired Student's t-test or the nonparametric Mann-Whitney rank-sum test. The possible role of confounding variables was investigated by Cox proportional hazards regression analysis, by introducing covariates (previous response to antiviral therapy, albumin, platelets, degree of portal hypertension, Fibroscan® value, age, bilirubin, renin and aldosterone) that were related to the analyzed event (SVR, PH and fibrosis regression) in a univariate analysis. p values of < 0.05 were accepted for multivariate analysis (the maximum number of variables included in the multivariate analysis was 1 per 5 outcomes). The contribution of each significant variable to the risk of reaching the endpoint was estimated by the relative hazard and corresponding 95% confidence interval (CI). The relationship between the sensitivity and specificity of the HVPG value and the response at week 8, week 12 and week 72 was evaluated using a receiver operating characteristic curve. We studied the correlation between Fibroscan®, HVPG and inflammatory biomarkers with SVR via Pearson's and Spearman's correlations. Calculations were performed with the SPSSv20 statistical package (SPSS Inc., Chicago, Illinois).

RESULTS

From a group of 20 patients eligible for the study, for whom a hemodynamic study was performed, four cases had a baseline HVPG below 6 mmHg, and they were excluded. The remaining 16 were finally included. In eight cases, SVR was obtained; four patients relapsed during the first month after treatment, and treatment was stopped early for non-response to lead in (one case), a decrease < 3 log at week 8 (two patients) and breakthrough at week 11 (one case) (Fig. 1).

During the study period, no patient presented clinical liver decompensation (variceal bleeding, ascites, encephalopathy). In one patient, complete portal thrombosis was detected by Doppler ultrasound at week 11; another patient was diagnosed with unicentric hepatocarcinoma three months after treatment (in both cases, a breakthrough was detected).

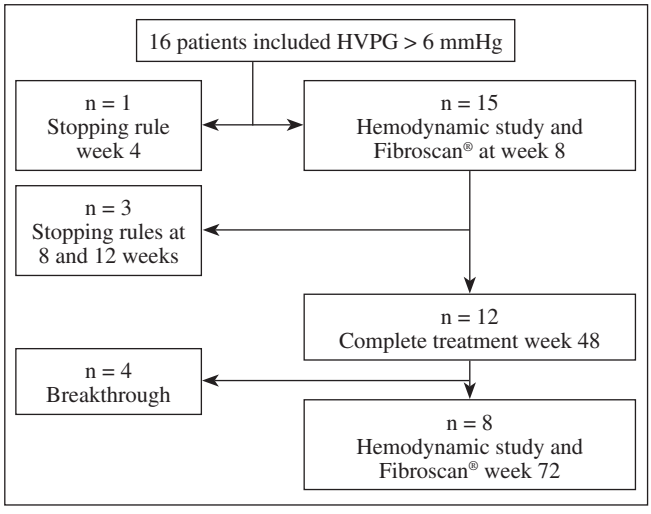

Fig. 1. Flowchart of the study.

Data analysis was performed according to the relationships of HVPG and non-invasive parameters of liver fibrosis with VL targets (decrease in VL > 3 log at treatment week 8 and SVR).

Baseline characteristics

As shown in table I, no statistically significant differences in baseline analytical, elastographic or hemodynamic parameters were found between patients who reached or did not reach SVR. By multivariate analysis we found that the previous null response (p = 0.049) and a high renin baseline value (p = 0.038) were strong baseline predictors of non-response (p < 0.05).

If we focus on the relationship with the stopping rules at weeks 8 and 72, we observe that HVPG, the platelet count and albumin did not reach statistical significance for predicting a decrease in VL at week 8 or SVR. The presence of a portal pressure gradient greater than 10 mmHg (eight patients) was not a predictor of viral response at week 8 or week 72. The baseline Fibroscan® value reached statistical significance as a response predictor at eight weeks of treatment (decrease in VL > 3 log; Fibroscan® 17.7 ± 8.9 kPa value *vs* 45.2 ± 22 kPa in non-responders, p = 0.08). Similarly, a cut-off of 11.6 kPa® had a sensitivity = 100%, 1-specificity = 0.895 and AUROC 0.974 (p = 0.031) for a VL decrease > 3 log at week 8.

Patient characteristics at week 8

There was a statistically significant decrease (p < 0.001) from baseline to week 8 in the levels of AST, ALT, GGT,

Table I. Baseline characteristic of the patients included in order to response to treatment

	SVR (n = 8)	*Non SVR (n = 8)*	*p value*
Sex (M/F)	2 (25)/6 (75)	4 (50)/4 (50)	0.320
Age (years)	53.13 ± 7.1	57.0 ± 8.8	0.350
Previous response to IFN (naïve, null, partial responder, relapse); n (%)	7 (87.5)/1 (12.5)/0	3 (37.5)/4 (50)/1 (12.5)	0.049
BMI (kg/m^2)	25.3 ± 6.2	26.23 ± 4.7	0.745
Child-Pugh (points)	5 ± 0.35	5.2 ± 0.46	0.554
MELD score	6.4 ± 1.16	6.6 ± 1.06	0.702
Hemoglobin (mg/dl)	14.17 ± 1.61	14.5 ± 1.35	0.669
Platelet count (x10^{-3})	143.88 ± 62.96	117.13 ± 55.15	0.381
Neutrophils (x10 $^{-3}$)	2,200.00 ± 851.89	2,556.25 ± 837.27	0.413
Albumin (mg/dl)	4.11 ± 3.7	3.91 ± 0.5	0.383
Bilirubin (mg/dl)	0.8 ± 0.44	0.92 ± 0.291	0.516
INR	1.05 ± 0.1	1.05 ± 0.1	0.963
Creatinin (mg/dl)	0.67 ± 0.06	0.77 ± 0.16	0.141
AST (UI/ml)	95.32 ± 39.4	72.13 ± 21.09	0.162
ALT (UI/ml)	127.63 ± 73.7	72.25 ± 29.15	0.068
HOMA	3.94 ± 2.83	4.04 ± 1.59	0.935
Viral load (log)	5.91 ± 1.15	6.09 ± 0.96	0.741
Genotype (1/1a/1b); n (%)	2 (25)/3 (37.5)/3 (37.5)	2 (25)/6 (75)	0.135
CC- IL 28 genotype; n(%)	2 (25)	2 (25)	0.237
Esophageal varices (clinically significant/small/ absent; n %)	0(0)/1 (12.5)/7 (87.5)	2 (25.2)/5 (62.3)/1 (12.5)	0.211
Portal hypertensive gastropathy (absent, present); n (%)	5 (62.3)/3 (37.5)	5 (62.3)/3 (37.5)	1
Renin (pg/ml)	9.4 ± 3.4	5.45 ± 3.5	0.038
Aldosterone (pg/ml)	52.71 ± 39.4	71.75 ± 61.8	0.497
Fibroscan® (Kpa)	18.78 ± 13.61	23.93 ± 15.97	0.499
Heart rate (bpm)	75.83 ± 19.53	73.33 ± 20.52	0.860
PAP (mmHg)	17.37 ± 2.9	19.0 ± 3.59	0.341
PWP (mmHg)	11.43 ± 2.39	11.37 ± 3.23	0.96
RAP (mmHg)	6.93 ± 2.1	7.12 ± 2.5	0.875
ICVP (mmHg)	10.0 ± 2.1	10.5 ± 3.42	0.637
FHVP (mmHg)	9.3 ± 2.68	11.25 ± 5.37	0.166
WHVP (mmHg)	19.93 ± 4.65	21.62 ± 5.37	0.513
HVPG (mmHg)	10.31 ± 4.46	10.87 ± 4.45	0.804
HVPG > 10 mmHg; n (%)	3 (37.5%)	5 (62.3%)	0.317

SVR: Sustained viral response; BMI: Body mass index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; HOMA: Homeostasis model assessment; PAP: Pulmonary arterial pressure; PWP: Pulmonary wedge pressure; RAP: Right auricular pressure; ICVP: Inferior vena cava pressure; FHVP: Free hepatic vein pressure; WHVP: Wedge hepatic vein pressure; HVPG: Portal pressure gradient.

hemoglobin, platelets, neutrophils, albumin, and bilirubin, as well as in the VL. No differences were found in the INR value (Table II).

Although in three patients the second hemodynamic study was not carried out (severe anemia, severe neutropenia and anxiety attack), a statistically significant HVPG decrease in all patients included in the study (Fig. 2) was found, except for one patient (non-response to lead in). However, no differences were found in the HVPG decrease based on viral response at week 8: 8.9 ± 4.58 mmHg (> 3 log) and 11.0 ± 3.35 mmHg (< 3 log), p > 0.05. Likewise, the HVPG percentage decrease at week 8 did not have a linear correlation with the decrease in VL.

As shown in figure 3, a statistically significant decline in the Fibroscan® value in all patients included in the study was observed (21.3 ± 14.5 *vs* 16.45 ± 6.18 kPa, p = 0.001). Similarly, when analyzing the Fibroscan® value at week 8 according to a decrease in VL > 3 log, we found that no patient with a week 8 Fibroscan® value > 24.8 kPa had a decrease > 3 log in VL at week 8 (AUROC = 1, S = 0.944 E 1, p = 0.023).

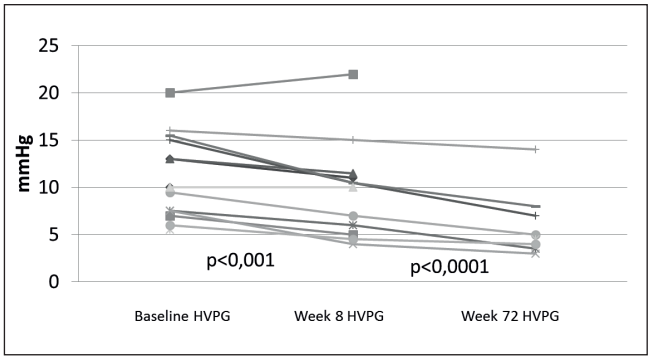

Fig. 2. Evolution of HVPG values during treatment (baseline and week 8 and week 72 HVPG values).

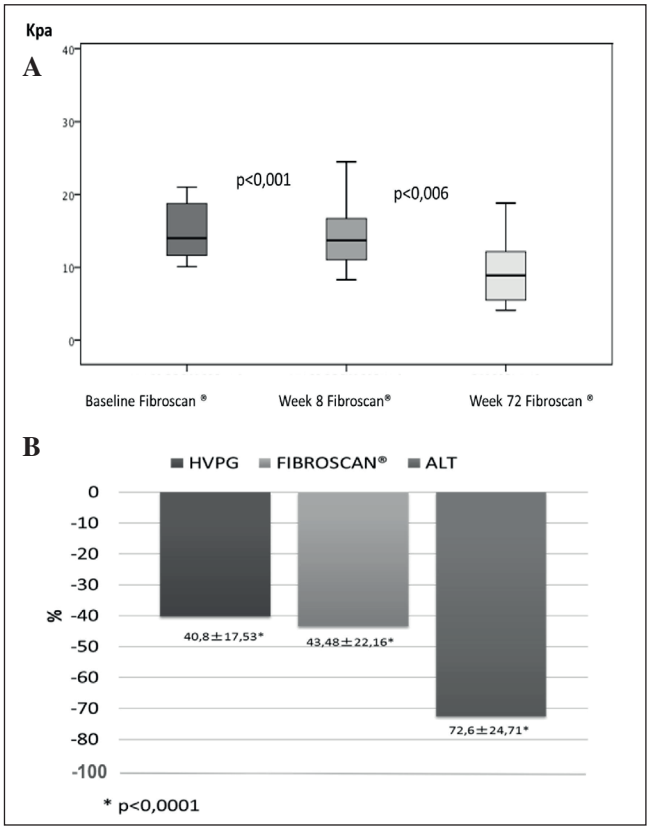

Fig. 3. A. Decrease of Fibroscan® in all patients at week 8 and 72 with respect to baseline. B. Percentage of decrease in HVPG, Fibroscan and ALT post-treatment. *p < 0.0001.

Table II. Baseline, week 8 and week 72 patients' characteristic

Value	*Baseline (median ± SD) n = 16*	*Week 8 (median ± SD) n = 16*	*p*	*Week 72 (median ± SD) n = 12*	*p**
AST (UI/ml)	83.75 ± 32.85	38.1 ± 11.87	*0.001*	28.8 ± 13.34	*0.001*
ALT (UI/ml)	99.94 ± 61.23	32.8 ± 11.5	*0.001*	25.01 ± 10.1	*0.001*
GGT (UI/ml)	99.38 ± 46.88	53.5 ± 24.8	*0.001*	37.5 ± 20.2	*0.001*
Bilirubin (mg/dl)	0.86 ± 0.46	0.769 ± 0.34	*0.03*	0.62 ± 0.49	*0.009*
Creatinin (mg/dl)	0.72 ± 0.13	0.77 ± 0.14	0.321	0.81 ± 0.15	0.234
Albumin	4.01 ± 0.44	3.72 ± 0.43	*0.001*	3.98 ± 0.34	0.254
INR	1.05 ± 0.11	1.07 ± 0.15	0.345	1.11 ± 0.099	*0.001*
Hemoglobin (g/dl)	14.33 ± 1.44	10.73 ± 1.3	*0.0001*	10.9 ± 1.96	*0.001*
Platelets (c* 10^3/dl)	130.1 ± 58.66	73.19 ± 34.3	*0.0001*	118.87 ± 73	*0.003*
Neutrophils (10^3)	2378.50 ± 836.45	1015.01 ± 408.08	*0.0001*	1325 ± 675	*0.001*
HOMA	3.99 ± 2.22	4.30 ± 3.3	0.567	3.76 ± 1.23	0.372
VL (UI/ml) $X10^6$	64.39 ± 176.52	7.65 ± 3.19	*0.0001*	Undetectable**	*0.00001*
VL Log	6.01 ± 1.1	1.15 ± 1.2	*0.0001*	Undetectable **	*0.00001*
Fibroscan®	21.36 ± 14.58	16.20 ± 6.44	*0.001*	9.5 ± 4.55**	*0.006*
HVPG (mmHg)	10.56 ± 4.31	9.4 ± 5.04 *	*0.001*	6.1 ± 3.61**	*0.0001*

p and p*: with respect to baseline; *n = 13; **n = 8. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma glutamyl transpeptidase; INR: International normalized ratio; HOMA: Homeostasis model assessment; VL: Viral load; HVPG: Portal pressure gradient.

A statistically significant correlation between the Fibroscan® value at week 8 and the HVPG value (Pearson correlation R^2 0.017 p = 0.05) was also found, and both (Fibroscan® and HVPG) had a strong correlation (bilateral Rho Spearman) with necroinflammatory activity (AST, R^2 0.590 p = 0.05; ALT, R^2 0.6 p = 0.005).

Patient characteristics at week 72

Twelve patients completed 48 weeks of treatment with a negative VL from week 12. However, in the first month after treatment, four breakthroughs were detected and were therefore excluded from the final hemodynamic study and Fibroscan® at week 72. Only the eight patients with SVR underwent the final hemodynamic study and Fibroscan®. The results were analyzed by intention-to-treat. In all 12 patients, there was a statistically significant decrease (p < 0.001) in the ALT, AST, bilirubin, INR, hemoglobin, neutrophil and platelet levels.

Compared to baseline, there was a significant decrease in HVPG in all patients with SVR (baseline HVPG [n = 8] 10.31 ± 4.46 mmHg *vs* HVPG-72 [n = 8] 6.1 ± 3.61 mmHg, p < 0.0001). The average decrease in HVPG during SVR was 40.8 ± 17.53%, achieving an HVPG < 6 mmHg in five patients (62.5%), an HVPG of 6-10 mmHg in two patients (25%) and an HVPG > 10 mmHg in one patient (12.5%) (Fig. 2).

We detected a significant decrease in the post-treatment Fibroscan® values compared to baseline in SVR patients: 21.36 ± 14.58 kPa *vs* 9.5 ± 4.55 kPa, p < 0.006. A significant regression of elastography was achieved (≤ 7.1 kPa) in three patients (37.5%); significant remaining liver fibrosis elastography (> 7.1 kPa) was observed in five patients (62.5%; one case > 12.5 kPa) (Fig. 3A).

Likewise, there was a statistically significant correlation between the HVPG value and Fibroscan® post-treatment (r^2 = 0.774, p = 0.024). Regarding necroinflammatory activity (ALT), we found a significant correlation with Fibroscan® (r^2 = 0.714, p = 0.046) but not with HVPG (r^2 0.551, p = 0.151). If we analyze the percentage decrease in these three parameters, as shown in figure 3B, the most clinically relevant decrease occurred in ALT (72.6 ± 24.71%), followed by Fibroscan® (43.48 ± 22,16) and HVPG (40.8 ± 17.53) (p < 0.0001).

Although patients with SVR had higher baseline renin values than patients without SVR, there was a statistically significant decrease compared to baseline 9.462 ± 3.4 pg/ml *vs* 6.42 ± 1.9 pg/ ml.

Serological parameters of portal hypertension. Inflammatory biomarkers

As expected, platelet count was found to be correlated with HVPG and Fibroscan® (r^2 = -0.548, p = 0.028 and r^2 = -0.67, p = 0.01, respectively). However, we found no relationship with inflammatory biomarkers. Additionally, we did not observe a significant difference in the baseline values of VCAM1, IL-6, IL-1β and IL-1Rα between SVR and non-SVR patients. VCAM1, IL-6, IL-1ß and IL-1Rα showed a statistically significant decrease (p < 0.0001), with a strong positive correlation with the renin/angiotensin axis (r^2 = 0.875, p = 0.016).

Safety and adverse effects

Seven patients (33%) developed an infection during treatment, with all seven cases occurring in the first 12 weeks (five respiratory infections, one case of cellulitis and one urinary tract infection). Only one of the patients developed severe pneumonia, requiring hospitalization. The development of infection was not related to the dose of interferon, neutrophil count, MELD score, or serum albumin or bilirubin level.

DISCUSSION

The first direct-acting antiviral agents (DAAs), boceprevir and telaprevir, allowed compensated cirrhotic patients to achieve an SVR success rate between 40% and 70%, whilst for previous treatment (PR), the response rate failed to reach 30%. This new cohort of patients who achieve SVR but have a prior diagnosis of cirrhosis with portal hypertension have raised a question whose answer was unknown until now: is the cure of infection associated with the healing of liver disease? Moreover, would it be reasonable to re-conceptualize the response in cirrhotic patients undergoing antiviral therapy to include histological and hemodynamic regression?

Previous studies with interferon or PR have shown (10,25,26) that achieving SVR in cirrhotic patients reduces all-cause mortality, mortality related to OLT, and the risk of hepatocellular carcinoma and liver failure (27-29). Despite achieving SVR, a key goal of therapy is to obtain histological and hemodynamic regression (9,30). Mallet et al. demonstrated that fibrosis progression is the most important risk factor for long-term liver-related events in 706 cirrhotic patients treated with PEG + RBV In this cohort of patients with a follow-up of 120 months, the percentage of liver-related events was near 40% in patients who did not achieve SVR or did not show fibrosis regression (including 17 patients with SVR) compared to 0% in patients with fibrosis regression and 15% in those with SVR (29). The portal pressure gradient is the most reliable, objective and reproducible portal pressure measurement, and is much better than liver biopsy for showing the severity of damage and the probability of decompensation during or after treatment. Although we are presently in the age of IFN-free therapies, HVPG would be able to select and highlight pos-

sible non-responders or those at high risk of decompensation under such treatment. A decrease in Fibroscan® value under antiviral treatment has been described previously (41), but we know that the correlation with HVPG < 10 mmHg is not optimal. Recently, in a cohort of non-cirrhotic patients treated with telaprevir-based TT, non-invasive fibrosis assessment by Fibroscan® was found to be useful for predicting SVR in prior partial or null responders, with a cut-off value of ≤ 10.0 kPa, AUROC 0.99. However, this result cannot be extrapolated because, in our cohort, advanced liver disease was required for inclusion (31).

In our study, we demonstrated that in a cohort of cirrhotic patients treated with triple therapy (TT), a 50% SVR rate was achieved, and a previous null response to interferon therapy was the only factor predictive of treatment failure, with no relation to HVPG, baseline Fibroscan® value, platelet count or inflammatory biomarkers.

The value of this study is that, for the first time, it demonstrated a decrease of greater than 40% in the portal pressure gradient in patients with portal hypertension treated with TT. This HVPG decrease is greater than the 25% decline described by Rincón et al. in cirrhotic patients treated with dual therapy (although the hemodynamic result was obtained in week 48). Additionally, complete regression of PH and, therefore, complete hemodynamic healing were achieved in more than half of the patients who reached SVR (HVPG < 6 mmHg in five patients, 62.5%); remaining mild portal hypertension was detected in two cases (25%) and clinically significant PH (HVPG > 10 mmHg) in one patient (12.5%). Likewise, complete fibrosis regression by Fibroscan® was achieved in three patients (37.4%), whereas significant post-treatment liver fibrosis was detected in 62.5%.

The mechanisms implicated in the decrease in HVPG may be related to the reversibility of increased intrahepatic vascular resistance in cirrhosis due to the impairment of intrahepatic nitric oxide (NO) and the pharmacological modification of stellate cell activity. The interaction between the anti-inflammatory effects of SVR and intrahepatic vascular resistance is unknown (33).

Unlike the study of Rincon et al., in which no relationship between the decrease in HVPG and fibrosis was identified, we found a marked reduction in both parameters. This may be due to the regression of fibrosis being slower than the regression of portal hypertension (our study was conducted until six months after stopping treatment, not immediately after its cessation). However, in our small cohort of patients, we found a much larger decrease in the portal pressure gradient which can be related to two aspects: first, a potential modulatory effect of the protease inhibitor (although there is no evidence for this); second, the previous study included patients with a subsequent relapse of HCV, wherein the regression of fibrosis and PH would be mild or absent.

We found a decrease in the Fibroscan® value and HVPG in all patients at week 8; however, this must be related to an improvement in necroinflammatory activity secondary to the decrease in VL and the immunomodulatory properties of IFN but not to the antifibrogenic effect. Another limitation of our study is the fact that correlation of liver stiffness is excellent for HVPG values less than 10 mmHg but it is not optimal for non-significant portal hypertension (17).

HVPG measurement is an invasive procedure and is not available in all hospitals; for these reasons, inflammatory markers that could be correlated with the pressure gradient were analyzed. The use of screening serum inflammatory biomarkers of HVPG is based on the fact that PH is related to liver injury and fibrosis, and these are associated with the activation of inflammatory pathways. IL-1β, a cytokine product of the inflammasome, its receptor IL-1Rα, and VCAM-1, a product of endothelial cells, have recently been published as robust inflammatory markers of PH (34,35). However, in our cohort of patients no correlation was found with these parameters. As expected, there was a decline in these values at week 8 and post-treatment, although without a relationship with HVPG.

Because our study was aimed at identifying and selecting patient candidates for treatment with triple therapy, we believe it is very important to complete the current rules for stopping at weeks 4, 8 and 12 to prevent prolonged treatment due to the risk of large numbers of side effects (4,36). Therefore, we are able to confirm that those patients at week 8 who achieved a decrease in VL > 3 log and a Fibroscan® < 24.8 kPa have an 80% probability of becoming VL negative at week 48, and thus must be considered as prognostic factors of a response. However, we must bear in mind that these patients also have a high probability of developing infections and lipopolysaccharide-binding protein (LBP, the protein involved in the acute-phase immunological response to gram-negative bacterial infections and endotoxaemia),

Table III. Inflammatory biomarkers values

Value	*Baseline (median ± SD) n = 16*	*Week 8 (median ± SD) n = 16*	*Week 72 (median ± SD) n = 8*
IL-1β pg/mL	1.26 ± 1.2	0.96 ± 0.365	1.95 ± 1.27
IL-1α pg/mL	1,146.53 ± 56.7	4,654.98 ± 11.5	3,695.15 ± 2,996.7
VCAM pg/mL	225,450.87 ± 307	225,201.8 ± 421	224,953.7 ± 39
TNFα pg/mL	215,028.8 ± 10,885.2	240,067.4 ± 76,189.2	236,759.7 ± 45,877.09
IL-6 pg/mL	45,685.3 ± 13,197.1	45,961.45 ± 14,322.2	45,987.4 ± 14,332

could have a key role in prevention of changes in the intestinal microbiota secondary to treatment that favors bacterial translocation (37-39). These results must be confirmed in a larger cohort of cirrhotic patients treated with the new interferon free regimens. These new treatments will allow SVR to be achieved in decompensated liver disease with severe portal hypertension; in these patients the evolution of the HVPG and fibrosis is unknown.

In our study, we conclude that complete response (hemodynamic response and SVR) in patients with HCV-related cirrhosis occurs in more than half of patients achieving SVR and must be another target in cirrhotic patients with SVR. Likewise, the risk of liver decompensation and hepatocellular carcinoma should be evaluated in future studies including patients treated with direct-acting antiviral agents (DDAs) (40).

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