Noninvasive Assessment of Fibrosis Regression after Direct-acting Antiviral Treatment in Hepatitis C Virus Patients

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ABSTRACT **Background:** New direct acting antiviral agent (DAA) therapies are associated with a high sustained virological response rate (SVR) in hepatitis C virus (HCV) patients. The understanding of the impact of SVR on fibrosis stage is limited.

Objectives: To determine the effect of treatment with the DAAs on long-term liver fibrosis stages, as determined by shear-wave elastography (SWE) or FibroTest©.

Methods: Fibrosis stage was determined at baseline and at 6-month intervals after end of treatment (EOT), using two-dimensional SWE or FibroTest©; APRI and FIB-4 scores.

Results: The study comprised 133 SVR12 patients. After a median follow-up of 15 months (range 6–33), liver fibrosis stage decreased by at least 1 stage in 75/133 patients (56%). Cirrhosis reversal was observed in 24/82 (29%). Repeated median liver stiffness SWE values in cirrhotic patients were 15.1 kPa at baseline (range 10.5–100), 13.4 kPa (range 5.5–51) at 6 months, and 11.4 kPa (range 6.1–35.8) at 12 months after EOT, $P = 0.01$. During the second year after EOT, no statistically significant differences in liver fibrosis stage in 12, 18, and 24 months were found. Splenomegaly was the only significant negative predictor of liver fibrosis regression during all time points of repetitive noninvasive assessment.

Conclusions: Following successful DAA treatment, the majority of our HCV patients with advanced fibrosis demonstrated significant improvement, as assessed by non-invasive methods. Advanced fibrosis stage was a negative predictor of fibrosis regression. Longer follow-up periods are required to further establish the impact of DAAs treatment in HCV patients with advanced fibrosis.

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KEY WORDS: chronic hepatitis C virus (HCV), direct acting antiviral agent (DAA), liver fibrosis regression, noninvasive assessment, shear-wave elastography (see editorial page XXX)

Data on the impact of direct acting antiviral agents (DAA)-induced SVR on fibrosis down staging is still scarce. Noninvasive tests are increasingly included in national and international guidelines. Such methods are used to assess liver fibrosis stage in order to make decisions before the initiation of antiviral treatment and to determine follow-up regimens after achieving SVR [1,2].

Commonly used noninvasive modalities for the assessment liver fibrosis include transient elastography (TE) and two-dimensional shear-wave elastography (SWE). Studies that have evaluated SWE accuracy in the assessment of liver fibrosis, demonstrated comparable or even superior results to TE in the diagnosis of fibrosis stage Metavir $F \geq 2$ or $F \geq 3$ and comparable results for the diagnosis of liver cirrhosis [3]. Similarly, SWE proved slightly superior to TE in diagnosis of intermediate fibrosis stages [3]. The significance of decreasing TE or SWE measurements following DAA therapy with regards to further management and surveillance is currently unknown.

Several fibrosis scores that are based on quantified serum levels of fibrosis biomarkers have been also developed and validated over the recent years. The patented FibroTest©, the non-patented fibrosis-4 score (FIB-4), and the aspartate aminotransferase-platelet ratio index (APRI) scores have been validated for chronic hepatitis C virus (HCV) fibrosis staging and show acceptable sensitivity and specificity, particularly in advanced fibrosis and cirrhosis [4].

The primary aim of our study was to determine the effect of treatment with the DAAs on long-term liver fibrosis stages, as determined by SWE or FibroTest©, as well as by the FIB-4 and APRI in a large cohort of patients with advanced fibrosis. In addition, we aimed to determine baseline predictive factors of post-SVR liver fibrosis regression and to prospectively assess the development of cirrhosis-related complications including the development of HCC following SVR.

PATIENTS AND METHODS

In this retrospective/prospective observational single center study, we used electronic health records data retrieved from the

HCV registry at the Sheba Medical Center. The study was conducted in accordance with the Declaration of Helsinki, current guidelines on good clinical practices, and local ethics and legal requirements. The protocol was approved by the institutional review board. All patients provided voluntary written informed consent before trial entry.

PATIENTS

The study comprised 165 genotype 1–4 HCV patients who achieved SVR for 12 consecutive weeks following DAA treatment. Patients were excluded when baseline fibrosis stage assessment was unavailable (n=14) or was assessed by liver biopsy (n=8) and in cases of baseline fibrosis stage F0 (n=10).

Finally, 133 SVR patients who had reached at least 6 months after EOT were enrolled. Pre-treatment noninvasive fibrosis stage was assessed by SWE (n=87), TE (n=18), or FibroTest© (n=28); FIB-4 and APRI methods. Patient's baseline clinical (including pre-treatment fibrosis assessment) and demographic data were collected retrospectively.

Post-treatment liver fibrosis stage was assessed prospectively at 6-month intervals by SWE (n=105) in patients with pre-treatment assessment by SWE or TE, and FibroTest© (n=28) in whom pre-treatment fibrosis was assessed by FibroTest©, as well as the determination of the APRI and FIB-4 scores.

Spleen size was determined by ultrasonography, computed tomography, or magnetic resonance imaging. The presence of esophageal varices in cirrhotic patients were assessed by gastroscopy. Spleen size of orthotropic liver transplantation (OLT) patients were excluded from statistical analysis (because splenomegaly persists after OLT).

For cirrhotic patients following successful DAA treatment, ultrasonography was performed, AFP levels were determined at 6-month intervals, and endoscopy was performed every 2 years.

ASSESSMENT OF LIVER FIBROSIS STAGE

Shear-wave elastography (SWE)

Liver stiffness (LS) was measured by SWE Aixplorer® (SuperSonic Imagine, Aix-en-Provence, France). For each patient, stiffness was defined as the mean of several SWE measurements [5].

SERUM FIBROSIS SCORES

FibroTest© (Biopredictive, Paris, France) is a commercially available algorithm that combines α2-macroglobulin, apolipoprotein A1, haptoglobin, total bilirubin, and γ-glutamyltranspeptidase, adjusted for age and gender [6].

The APRI [6] and the FIB-4 (age [year] × AST [U/L]/ platelets [109/L] × ALT [U/L]) [6] were also calculated from laboratory values.

Cutoff values for significant liver fibrosis and cirrhosis were adopted from the European Association for the study of the Liver (EASL) guidelines for non-invasive assessment of liver fibrosis [2]. Fibrosis improvement was defined as a ≥ 1-point change in fibrosis stage.

ANTIVIRAL THERAPY

Patients with chronic HCV infection were treated with DAAs according to the EASL recommendations [5]. However, between September 2014 and November 2016, the Israel health authorities had limited access to some DAA combination therapies and DAAs treatment access was limited to patients with fibrosis stage F3–4. Treatment duration was 12–24 months, with/without ribavirin, depending on genotype, liver fibrosis stage and the selected DAA regimen. The DAA regimen was as per physician's discretion.

SVR was defined as undetectable HCV RNA level 12 weeks after end of treatment (EOT) (COBAS 4800; Roche Diagnostics, Indianapolis, Indiana, USA).

STATISTICAL ANALYSIS

Baseline characteristics were compared between patients with different stages of fibrosis (F4 with F3, F4 with F1–2 and F3 with F1–2). Continuous variables were expressed as median and categorical variables as a percentage. Categorical variables were compared using the chi-square and Fisher's exact tests. Continuous variables were compared using the Mann–Whitney U test. When considering the continuous variables for dichotomous analysis, cutoff values were determined by receiver operating characteristic (ROC) analysis. The difference in the changes of continuous variables during follow-up was assessed using the paired Wilcoxon signed-rank test. Linear regression analysis was used to determine which baseline variables were associated with the change in fibrosis stage. For multivariate analysis, all variables with $P < 0.1$ were included in the model. A ROC analysis was performed to evaluate the accuracy of prediction of fibrosis regression. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant. Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software, version 23 (SPSS, IBM Corp, Armonk, NY, USA).

RESULTS

PATIENT BASELINE CHARACTERISTICS

Patient baseline characteristics and laboratory parameters are summarized in Table 1. One patient had HIV co-infection and none of the patients were co-infected with hepatitis B. Seven of 133 include patients who underwent orthotropic liver transplantation (OLT) and developed HCV recurrence in liver graft before DAA treatment.

Seventy-five of the patients (56%) were treatment-naïve, 103 (77.4%) were genotype 1B. Eighty-two (61.7%) had compen-

sated cirrhosis with Child-Pugh score A; F3 36 (27.1%); F2 12 (9%); and F1 3 (2.3%). Median LS SWE value prior to therapy was 15.1 kPa (range 10.5–100) in the F4 group, 10 kPa in the F3 group (range 9–11.6), 8.3 kPa in the F2 group (range 7.5–9.0), and 7.1 kPa in the F1 group (range 7.0–7.2). Median FibroTest© value prior to therapy was 0.8 (range 0.76–0.94), 0.68 (range 0.66–0.73), and 0.52 for F4, F3, and F2 patients, respectively

Sixty-four patients received ombitasvir/paritaprevir/ritonavir with dasabuvir, 2 patients received sofosbuvir/ ribavirin, 6 patients received elbasvir/grazoprevir, 7 patients received sofosbuvir/daclatasvir, and 3 patients received ledipasvir/sofosbuvir [Table 1].

FIBROSIS REGRESSION

After a median follow-up period of 15 months (range 6–33 months), liver fibrosis stage, which was assessed by SWE or FibroTest©, decreased by at least 1 stage in 75 patients (56%). Of the 82 cirrhotic patients, 24 (29.3%) demonstrated regression in liver fibrosis, 10 (12.2%) of whom regressed to F3, 9 (11%) to F2, 2 (2.4%) to F1, and 3 (3.7%) to F0 fibrosis stage [Table 2].

Among patients with F3 (n=36); fibrosis stage worsened to F4 in one (2.8%), remained stable in 10 patients (27.8%), and decreased by at least one stage in 25 patients (69%) [Table 2]. Among patients with fibrosis stage F2 (n=12), no change in fi-

Table 1. Patiens baseline demographics and laboratory parameters

	All n=133	F4 n=82	F3 n=36	F1-2 n=15	*P* value F4 vs. F1-3	*P* value F3 vs. F1-2	*P* value F4 vs. F3
Age, years (range)	60 (28–82)	61 (28–82)	59 (29–80)	59(44–78)	0.4	0.6	0.3
Male, n (%)	73 (54.9)	43 (52.4)	20 (55.6)	10(66.7)	0.5	0.46	0.76
Body mass index (kg/m2)	27 (17–42)	28 (19–42)	26 (18–37)	28 (17–35)	0.1	0.3	0.053
Dyslipidemia, n (%)	29 (22)	20 (24.4)	7 (19.4)	2 (13.3)	0.3	0.7	0.56
Hypertension, n (%)	66 (50)	48 (58.5)	14 (38.9)	4 (26.7)	0.009	0.3	0.049
Diabetes, n (%)	39 (29)	29 (35.4)	9 (25)	1 (6.7)	0.052	0.1	0.27
Esophageal varices, n (%)	12 (9)	12 (14.6)	0	0	0.004	0.19	0.006
Splenomegaly, n (%)	35 (26)	31 (37.8)	3 (8.3)	1 (6.7)	0.001	0.9	0.005
Treatment naïve, n (%)	75 (56)	35 (42.7)	16 (44.4)	7 (46.7)	0.8	0.88	0.86
Liver transplantation, n (%)	7 (5.2)	6 (7.3)	1 (2.8)	0	0.17	0.5	0.3
Genotype, n (%)					0.17	0.9	0.3
1b	103(77.4)	66 (80.5)	26 (72.2)	11 (73.3)			
1a	15 (11.3)	8 (9.8)	5 (13.9)	2 (13.3)			
2	4 (3)	4 (4.9)	0	0			
3	7 (5.3)	3 (3.7)	3 (8.3)	1 (6.7)			
4	4 (3)	1 (1.2)	2 (5.6)	1 (6.7)			
Creatinine (mg/dl)	0.8 (0.5–2.3)	0.8 (0.5–2.3)	0.9 (0.5–1.5)	0.8 (0.5–1.2)	0.7	0.77	0.7
Albumin (g/dl)	4.1 (2.6–4.9)	4.0 (2.6–4.8)	4.2 (3.5–4.9)	4.2 (3.5–4.8)	0.009	0.78	0.014
Bilirubin (mg/dl)	0.8 (0.1–3.2)	0.8 (0.3–3.2)	0.7 (0.1–1.6)	0.7 (0.5–2.6)	0.01	0.44	0.008
ALT (U/L)	84 (11–454)	93 (25–454)	69 (11–210)	62 (30–141)	0.01	0.36	0.057
AST (U/L)	73 (24–272)	85 (31–272)	60 (24–131)	58 (34–144)	0.006	0.75	0.018
Platelets (109/L)	155 (49–340)	140 (49–260)	200 (92–340)	177 (102–314)	> 0.0001	0.6	> 0.0001
INR	1	1.0 (0.8–1.2)	1 (0.9–1.1)	0.9 (0.9–1.2)	0.057	0.5	0.26
Fibrosis stage SWE, kPa	11.5 (7–100)	15.1 (10.5–100)	10 (9–11.6)	8.3 (7.0–9.0)	> 0.0001	> 0.0001	> 0.0001
FibroTest©	0.77 (0.52–0.94)	0.8 (0.76–0.94)	0.68 (0.66–0.73)	0.52	0.003	0.5	0.008
APRI	1.1 (0.2–7.2)	1.6 (0.5–7.2)	0.8 (0.2–2.6)	0.8 (0.3–2.6)	> 0.0001	0.9	> 0.0001
FIB-4	3 (0.8–18.9)	3.7 (1.1–18.9)	2.2 (0.8–6.1)	2.0 (1.0–6.6)	> 0.0001	0.9	> 0.0001

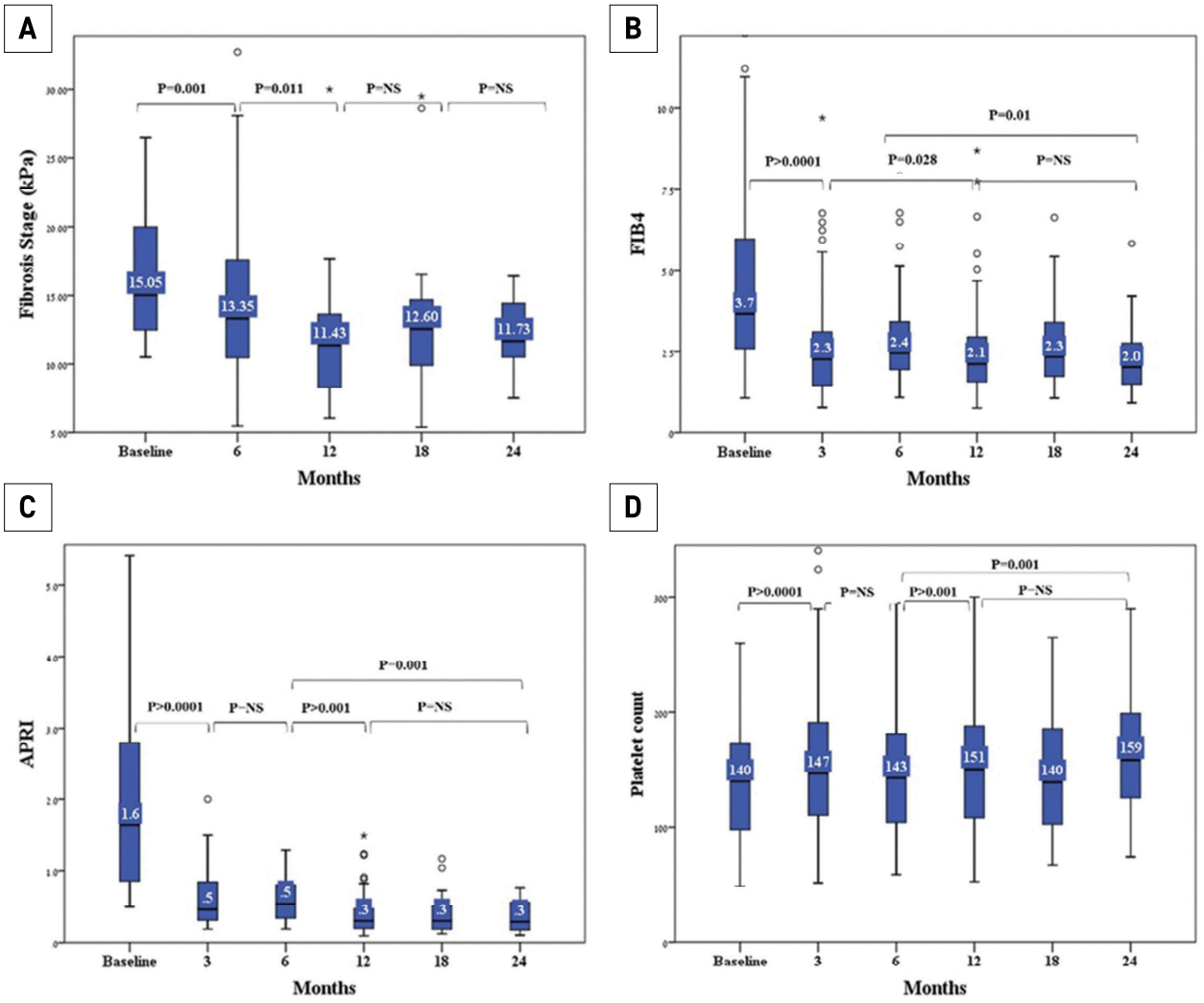
Continuous variables are expressed as median values (range)

ALT = alanine-aminotransferase, APRI = aspartate aminotransferase-platelet ratio index, AST = aspartate aminotransferase, FIB-4 = fibrosis-4 score, INR = International Normalized Ratio, SWE = shear-wave elastography

Figure 1. Baseline versus post-treatment **[A]** LS SWE, **[B]** FIB-4, **[C]** APRI and **[D]** platelet counts in cirrhotic patients following SVR

APRI = aspartate aminotransferase-platelet ratio index, LS SWE = liver stiffness measured by shear-wave elastography, FIB-4 = Fibrosis-4 Score, SVR = sustained virologic response

Box plots show comparison of median values pre-and post-treatment of LS SWE **[A]**, FIB-4 **[B]**, APRI **[C]**, and platelet counts **[D]** every 6 months after end of treatment. Values were compared using Mann–Whitney U test



brosis stage was noted in 4 patients (33.3%), and improved by at least one stage in 8 patients (66.7%). Among patients with fibrosis stage F1 (n=3), fibrosis stage worsened to F2 in one (33.3%) and showed no change in 2 patients (66.7%). None of the patients with F1–2 experienced fibrosis stage worsening [Table 2].

REGRESSION OF FIBROSIS STAGE IN CIRRHOTIC PATIENTS AFTER ACHIEVING SVR

We performed additional evaluation of regression of fibrosis stage in cirrhotic patients after achieving SVR. Repeated median LS SWE values decreased significantly from 15.1 kPa (range 10.5–100) at baseline to 13.4 kPa (range 5.5–51) (P = 0.001) 6 months after EOT. A significant decrease was measured 6 months after, with a median LS SWE values of 11.4 kPa (range 6.1–35.8) 12 months after EOT (P = 0.011). Median LS SWE value 18 months after EOT was 12.6 kPa (range 5.4–36) and 24 months after EOT was 11.5 kPa (range 5.2–16.4) [Figure 1A].

Repeated median FT values decreased from 0.77 (range 0.52–0.94) at baseline to 0.63 (range 0.34–0.94) 12 months after EOT, P = 0.03 [Table 2].

Repeat calculation of APRI and FIB-4 score every 6 months after EOT are presented in Figure 1 and Table 2. The decrease from baseline median APRI scores 1.6 (range 0.5–7.2) was statistically significant 3 months after EOT 0.5 (range 0.2–2.0), P

Table 2. Changes in laboratory parameters and noninvasive fibrosis assessment results

	Fibrosis stage kPa, median (range)	FibroTest©	APRI median (range)	FIB-4 median (range)	AST (IU/ml) median (range)	ALT (IU/ml) median (range)	Platelets K × 10^9) median (range)
Baseline	15.1 (10.5–100)	0.77 (0.52-0.94)	1.6 (0.5–7.2)	3.7 (1.1–18.9)	85 (31–272)	93 (25–454)	140 (49–260)
Months after EOT							
3			0.5 (0.2–2.0)	2.3 (0.8–9.7)	27 (16–65)*	25 (9–66)*	147 (52–340)
6	13.4 (5.5–51)		0.5 (0.2–1.3)	2.4 (1.1–8.1)	26 (20–57)	23 (8–75)	143 (59–295)
12	11.4 (6.1– 35.8)	0.63 (0.34–0.94)	0.3 (0.1–1.5)	2.1 (0.8–8.7)	26 (13–55)	25 (8–91)	151 (53–300)
18	12.6 (5.4–36)		0.3 (0.1–1.2)	2.3 (1.1–6.6)	27 (17–55)	25 (12–56)	140 (67–265)
24	11.5 (5.2–16.4)		0.3 (0.1–0.8)	2.0 (0.9–5.8)	28 (15–59)	25 (8–68)	159 (74–290)

Continuous variables are expressed as median values (range)
*Wilcoxon two related sample test; ALT and AST baseline vs. 3 months, $P > 0.0001$
ALT = alanine-aminotransferase, APRI = aspartate aminotransferase-platelet ratio index, AST = aspartate aminotransferase, EOT = end of treatment, FIB-4 = fibrosis-4 score

> 0.0001. Median APRI score at 6 months after EOT was 0.5 (range 0.2–1.3) and 0.3 at 24 months EOT (range 0.1–0.8), $P = 0.001$. Median FIB-4 scores at 6 and 24 months EOT were 2.4 (range 1.1–8.1) and 2.0 (range 0.9–5.8), respectively ($P = 0.01$).

For understanding which parameter included in the APRI or FIB-4 scores was associated with its decrease, a separate analysis of changes of aspartate aminotransferase (AST), alanine-aminotransferase (ALT), and platelets was performed [Figure 1] [Table 2]. Median AST levels decreased from 85 IU/ml at baseline to 27 IU/ml 3 months after EOT ($P > 0.0001$). Median ALT levels decreased from 93 IU/ml at baseline to 25 IU/ml 3 months after EOT ($P > 0.0001$) and did not change significantly during follow-up. In contrast, platelets count increased significantly from a baseline median platelets count of 140 × 109/L (range 49–260) to 147 × 109/L (range 52–340) at 3 months ($P = 0.004$). Three months thereafter, median platelets count was 143 × 109/L (range 59–295) and was 159 × 09/L (range: 74–290) at 24 months $P = 0.011$. These findings can explain the progressive improvement in APRI and FIB-4 scores during the 2-year follow-up, despite normal level of transaminases.

BASELINE PREDICTORS OF FIBROSIS REGRESSION IN CIRRHOTIC PATIENTS

Prediction of fibrosis stage improvement at 6 months after EOT

At 6 months after EOT, 54 cirrhotic patients underwent fibrosis stage assessment (2 by FibroTest© and 52 by SWE). In 15 of the 54 patients (28%), fibrosis stage improved by at least 1 stage. Among patients who showed no improvement in fibrosis stage (un-improved) at 6 months after EOT, 63% had baseline splenomegaly vs. the 7% incidence among improved patients ($P > 0.0001$, odds ratio [OR] 0.46, 95% confidence interval [95%CI] 0.26–0.83, Pearson's $R = 0.64$). Esophageal varices were detected at baseline in 10 (37%) of the un-improved patients, compared to none in improved patients ($P = 0.061$, OR 0.7, 95%CI 0.5–0.9). Baseline median platelets count was significant higher in improved patients (162 × 109/l), as compared to un-improved patients (112 × 109/l); $P = 0.001$. Median APRI score was 1 vs. 2.2 ($P > 0.0001$) and FIB-4 score was 4.8 vs. 2.6 ($P = 0.001$) in improved and un-improved patients, respectively [Table 3].

The area under the curve (AUC) for the prediction of fibrosis improvement using *baseline platelets* count was 0.8 ($P = 0.001$). Platelets count at a cutoff of 150 K × 109/L predicted improvement of fibrosis stage at 6 months with sensitivity of 77% and specificity of 73%. Prediction of improvement of fibrosis stage at 6 months after EOT using baseline APRI score with cutoff < 1.7 had an AUC of 0.73 ($P = 0.01$) sensitivity of 93% and specificity of 62%. The AUC for the prediction of fibrosis stage improvement by this same time point, using baseline FIB-4 score was 0.74 ($P = 0.007$). FIB-4 score cutoff < 3.7 predicted improvement of fibrosis stage at 6 months with sensitivity of 87% and specificity of 62%.

The baseline median LS in SWE values in improved patients 6 months after EOT versus un-improved were 11.7 kPa (range 10.5–17.3) and 18.1 kPa (range 10.6–50), respectively ($P = 0.001$). The AUC for the prediction of fibrosis stage improvement at 6 months after EOT using baseline LS SWE was 0.83 ($P = 0.001$). A baseline LS SWE cutoff < 15.3 kPa predicted improvement of fibrosis stage at 6 months with a sensitivity of 92% and specificity of 61%. Only two patients were included in the FibroTest© group, precluding further analyses of this nature.

Univariate analysis showed no association between improvement in fibrosis stage and age, gender, co-morbidities (diabetes mellitus, hypertension, dyslipidemia, BMI), previous treatment with interferon, genotype of hepatitis C, baseline laboratory parameters as creatinine, albumin, or bilirubin and transaminases levels. Multivariate analyses showed independent associations between improvement of fibrosis stage in cirrhotic patients 6 months after EOT and baseline platelets count, FIB-4 and APRI scores, splenomegaly, and baseline LS measured by SWE [Table 3].

Table 3. Baseline predictors of fibrosis regression 6 months after end of treatment in cirrhosis patients

Covariant	Improved	Un-improved	Univariate analyses		Multivariate analyses	
	n=15	n=39	P value	Odds ratio, 95% confidence interval	P value	Odds ratio, 95% confidence interval
Hypertension	6 (40)	26 (66.7)	0.074	3 (0.9–10)	0.9	
Splenomegaly	1 (7.1)	22 (62.9)	0.0001	22 (2.6–188)	0.015	0.06 (0.06–0.6)
LS SWE<15.3 kPa	11.7 (10.5–17.3)	18.1 (10.6–50)	0.001	18.5 (2–163.5)	0.015	20 (1.8–227)
Platelets, cutoff <150 K*(109/l)	162 (120–219)	112 (49–235)	0.001	0.1(0.03–0.4)	0.018	0.14 (0.03–0.7)
APRI score, cutoff >1.7	1 (0.5–2)	2.2 (0.5–7.2)	0.0001	12 (2.7–194.5)	0.02	14 (1.5–138)
FIB-4 score, cutoff >3.7	2.6 (1.5–5.4)	4.8 (1.1–18.9)	0.001	10.7 (2.1–54.5)	0.049	0.15 (0.02–0.9)

Continuous variables are expressed as median values (range)
APRI = aspartate aminotransferase-platelet ratio index, FIB-4 = fibrosis-4 score, LS = liver stiffness, SWE= shear-wave elastography

Prediction of fibrosis stage improvement at 12 months after EOT

At 12 months after EOT, fibrosis stage was assessed in 60 patients (19 by FibroTest© and 41 by SWE). Multivariate analyses performed to identify baseline predictors of fibrosis regression at this time point singled out splenomegaly as the only independent predictor of fibrosis regression one year after EOT.

Prediction of fibrosis stage improvement at 18 months after EOT

At 18 months after EOT, 22 patients were assessed for fibrosis stage (all by SWE). Of these, 9 (41%) showed improved fibrosis stage. When comparing improved versus un-improved patients, baseline splenomegaly was detected in 2 (25%) and 10 (83%), respectively ($P = 0.009$). Esophageal varices were detected in 0 and 5 (50%), respectively.

The AUC for the prediction of fibrosis improvement at 18 months after EOT using baseline body mass index (BMI) was 0.8 ($P = 0.019$). Cutoff BMI > 28 kg/m2predicted fibrosis stage improvement at 18 months with a sensitivity of 89% and specificity of 77%. The AUC for the prediction of fibrosis stage improvement at 18 months after EOT using baseline bilirubin was 0.8 ($P = 0.03$). Baseline bilirubin 0.83 mg/dl cutoff predicted improvement of fibrosis stage at 18 months with a sensitivity of 75% and specificity of 66%. No other assessed clinical or demographic characteristics predicted improvement in fibrosis stage at 18 months. Multivariate analysis could not be performed due to the small number of patients at both the 18 months and 24 months of follow-up points.

Surrogate markers for predicting fibrosis improvement in SVR cirrhotic patient 12 months after EOT

Platelets count > 152 × 109/L 12 months after EOT predicted fibrosis improvement in cirrhotic patients assessed by SWE 1 year after EOT (AUC = 0.78, sensitivity 73% and specificity 77%, $P = 0.008$; Pearson's correlation $r = -0.405$, $P = 0.024$). No predictors of liver fibrosis regression assessed by FibroTest© were identified.

COMPLICATIONS

Two of the 82 cirrhotic patients (2.4%) developed cirrhosis-associated complications during the follow-up period. Neither patient showed fibrosis stage improvements. One patient developed upper gastrointestinal bleeding due to esophageal varices 2 years after EOT and the second developed de-novo hepatocellular carcinoma 7 months after EOT. None of the cirrhotic patients with fibrosis stage improvement developed liver-related complications. No case of death or need for liver transplantation was reported during the follow-up period.

DISCUSSION

Data on the impact of DAA-induced SVR on fibrosis down staging is still scarce. Use of SWE or biomarkers scores is becoming the basis for further patient surveillance and management after eradication of HCV. To the best of our knowledge, this is the first study that evaluated prospectively collected changes of liver stiffness assessed by repetitive SWE after successful, INF-free DAA treatments in a large cohort of 133 patients with advanced fibrosis stage (F3 4 88.8%), over an extended follow-up (median 15 months) period.

In the majority of the patients, fibrosis stage decreased (overall 56%) by 1 stage. Among them were 44% of the compensated cirrhosis patients, and in 85% of the patients with F3. More specifically, liver stiffness as assessed by SWE decreased significantly during the first year after EOT and remained stable during the second year. The fibrosis stage assessed by FibroTest©, also decreased significantly one year after EOT compared to the baseline values.

Bachofner et al. [7] reported rapid regression of LS assessed by TE, performed 15 weeks and 40 weeks post-treatment. A recent study also revealed significant improvement from baseline LS assessed by TE at EOT and 12 months post-treatment [8]. While there was some improvement in the LSM score between baseline and EOT, there was continued improvement between

the EOT and 12 months post-treatment ($P > 0.0001$) [8]. These findings are consistent with our observation of rapid improvements in LS during the first year after EOT in patients with advanced fibrosis stage who achieved SVR following DAAs treatment. In addition, in consistence with previous studies (14), the APRI and FIB-4 scores improved after successful DAA treatment. However, we also noted for the first time that the fibrosis stage remained stable during the second year post-SVR.

The effect of SVR on portal pressure was investigated in patients with SVR who also underwent HVPG and TE after IFN-free therapy [9]. HVPG decreased significantly across all cirrhotic patients with a median time between EOT and HVPG measurement of 114 days [9]. Lens and colleagues [10] also reported decreased HVPG from baseline 15 mmHg to 13 mmHg 24 weeks after SVR. Multivariate analysis demonstrated that previous acute variceal bleeding and the presence of thrombocytopenia were independently associated with increased HVPG after SVR. These finding are consistent with our observation that patients with more advanced cirrhosis, who had signs of portal hypertension (low baseline platelets count, baseline splenomegaly, esophageal varices) had high baseline LS SWE measurements and were associated with negative prediction of fibrosis regression.

In the second year of follow-up, liver fibrosis did not change significantly; however, when analyzing a subgroup of patients that had liver fibrosis assessments 18 months after EOT, we found that cirrhotic patients with BMI < 8 kg/m2 had a significantly higher chance of improved liver fibrosis stage. Other compounds of metabolic syndrome such as hypertension, diabetes mellitus or dyslipidemia had not significant influence on liver fibrosis improvement.

We have also noted that platelet counts increased significantly during the median follow-up period of 15 months, findings that have not been reported previously. We suggest that the measurement of platelets (one year after EOT) can be used as a surrogate marker to predict fibrosis stage improvement, with a cutoff 152× 109/L, with sensitivity 73 % and specificity 77%.

LIMITATIONS

The main limitations of our study are the not completely standardized time points of performing SWE, FibroTest©, APRI, and FIB-4 prior to and after treatment. In addition, the follow-up period was relatively short (median 15 months), ranging from 6 to 33 months. Further regression beyond this time span is expected based on previous long-term evaluations of patients following INF-based treatment [11,12].

CONCLUSIONS

Following successful DAAs treatment the non-invasive methods used here showed that the majority of HCV patients with advanced fibrosis stage benefited from significant improvements in liver fibrosis stage. Thus, SWE can be used as a reliable fibrosis stage surveillance tool following SVR. Platelets count can be used when other noninvasive methods are unavailable for the prediction of fibrosis regression. Longer follow-up periods and a larger cohort are required to further characterize the impact of DAAs treatment in HCV patients.

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As freely as the firmament embraces the world, or the sun pours forth impartially his beams, so mercy must encircle both friend and foe.

Johann Christoph Friedrich von Schiller (1759–1805), German playwright, poet, dramatist, and philosopher