

Liver Fibrosis Regression on Acoustic Radiation Force Impulse Elastography after Successful Direct-Acting Antiviral Therapy: 1-Year Follow-Up

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ABSTRACT

AIM: Acoustic radiation force impulse (ARFI) is an ultrasound elastography that delineates liver stiffness measurement (LSM) non-invasively. Data on the utility of ARFI in monitoring hepatic fibrosis evolution after hepatitis C virus (HCV) cure are still lacking. This study was designed to investigate the serial changes of ARFI-LSM and serum-based fibrosis scores in HCV-infected patients who achieved sustained virologic response (SVR) after direct-acting antivirals (DAAs) therapy.

MATERIAL AND METHODS: This study included 174 treatment-naïve HCV-infected patients who achieved SVR after DAAs. ARFI was performed in addition to fibrosis-4 (FIB-4) and aspartate aminotransferase-to-platelet count ratio index (APRI) scores for patients

at baseline, end of therapy (EOT), 12, 24 and 48 weeks after EOT.

RESULTS: Serum aminotransferases declined significantly overtime till 12 weeks after the EOT. Platelet count showed continuous significant increase over the follow-up period. Both FIB-4 and APRI scores demonstrated a continuous significant decline between the baseline and 48 weeks after EOT, except the period between EOT and 12 weeks after EOT when there was no significant change observed in FIB-4. There was a continuous significant decline of ARFI-LSM from the baseline over the follow-up period. In non-cirrhotic patients, the higher baseline ARFI-LSM was an independent predictor of fibrosis regression at 48 weeks after EOT. Absent portal hypertension and compensated liver disease at baseline were associated with ARFI-LSM improvement at 48 weeks after EOT in cirrhotic patients.

CONCLUSIONS: Continuous improvement in LSM after HCV eradication occurred due to resolution of liver injury and fibrosis regression. ARFI is a feasible and promising non-invasive method for hepatic fibrosis assessment.

Key words: Hepatitis C Virus; Fibrosis regression; Direct-acting antivirals; Acoustic radiation force impulse elastography; Liver stiffness

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INTRODUCTION

Hepatitis C virus (HCV) infection is a major global public health issue that represents one of the main causes of chronic liver disease worldwide^[1]. Chronic hepatitis C (CHC) has a long-term impact that ranges from minimal damage to extensive fibrosis and cirrhosis, which is accompanied with increased risk of hepatocellular carcinoma (HCC) development and liver failure^[2]. Cirrhosis represents the final stage of fibrosis in almost every chronic liver

disease^[3]. The stage of hepatic fibrosis is the principal predictor of HCV-induced liver disease progression which varies widely^[4]. It has since been demonstrated that fibrosis may revert in some cases of a wide spectrum of chronic liver diseases once the causative injury is eliminated, however definitive data is lacking^[5]. The objective of CHC treatment is to achieve a sustained virologic response (SVR) which is considered equivalent to cure in most cases^[6]. Long-term follow up studies indicated that achievement of SVR after interferon (IFN)-based therapy results in significant histological improvement and resolution of the extent of hepatic fibrosis that in turn reduces the risk of liver-related complications^[7,8]. The treatment of HCV has been recently evolved with the development of direct-acting antivirals (DAAs) which allowed for simplified and shortened regimens with increased tolerability and efficacy and wider therapeutic window^[9].

Assessment of inflammatory activity and fibrosis stage prior to HCV therapy is a well-known common practice^[9]. Since liver fibrosis remains despite its gradual resolution after SVR, the serial estimation of the extent of fibrosis is therefore desirable^[10]. Liver biopsy had long been the gold standard for diagnosis and assessment of the extent of liver disease in CHC, however, it is no more considered a perfect methodology because of its invasiveness and somehow questionable accuracy which led to limit its usefulness^[11]. Non-invasive methods have been currently developed for pre-treatment evaluation of liver disease which pose fewer risks, are better tolerated by patients and are suitable for longitudinal study of changes in fibrosis in HCV-infected patients^[12]. Despite their advantages over liver biopsy, the use of non-invasive methods to evaluate the evolution of long-term fibrosis and cirrhosis after treatment has not been sufficiently validated, and these tests capture both the influence of liver inflammation and injury, as well as fibrosis^[12]. A multitude of serum-based fibrosis scores have been developed and validated over the recent years. Fibrosis-4 (FIB-4) index and aspartate aminotransferase-platelet ratio index (APRI) scores have been demonstrated to be accurate in pre-treatment staging of chronic hepatitis and prediction of hepatic fibrosis in HCV-infected patients with acceptable sensitivity and specificity particularly in advanced fibrosis and cirrhosis^[13].

Liver stiffness measurement (LSM) is correlated with liver fibrosis and cirrhosis, as determined by liver biopsy, and its values are strongly correlated with the METAVIR fibrosis stages^[14]. A substantial overlap of LSM values was however observed between lower fibrosis stages, which means LSM performs better for detection of cirrhosis than for detection of significant fibrosis^[12,14]. LSM was demonstrated in clinical studies as a useful tool for assessment of the clinical effects of HCV treatment and evaluation of liver fibrosis post-SVR, although stiffness can also be influenced by liver inflammation and injury^[14,15]. Acoustic radiation force impulse (ARFI) is an elastography technique that is integrated in conventional ultrasonography equipment. It allows quantitative assessment of liver elasticity through providing measurements of shear wave velocity within a small measurement volume whose localization is monitored by real-time B-mode ultrasound^[14]. Several recent reports and meta-analyses have demonstrated that ARFI elastography for LSM is a valuable method to assess liver fibrosis in chronic viral hepatitis, such as hepatitis B or C virus-infected patients, and non-alcoholic fatty liver disease^[17-19]. It is still debated to what extent fibrosis is reversible and whether there is a point of no return beyond which HCV cure may be of uncertain clinical benefit^[20].

Therefore, the current study was designed to investigate the serial changes of ARFI-LSM and serum-based fibrosis scores (APRI and FIB-4) in HCV-infected patients who achieved SVR after DAAs therapy.

MATERIAL AND METHODS

Patients and study design

This is a prospective observational study conducted at Hepatobiliary Unit, Alexandria Main University Hospital in the time period between March 2018 and January 2020. A total of 174 treatment-naïve HCV-infected patients with positive test for HCV antibody and detectable serum HCV RNA were included. Patients who had hepatitis B virus (HBV) or human immunodeficiency virus co-infection, other known causes of chronic liver disease, concomitant schistosomiasis, uncontrolled diabetes mellitus, advanced cardiopulmonary or renal diseases or any kind of malignancy were excluded. Eligible patients were treated with IFN-free DAAs regimens according to the clinical recommendations on treatment of hepatitis C^[9]. SVR was defined as undetectable serum HCV-RNA 12 weeks after the end of therapy (EOT). All patients who achieved SVR12 were medically followed till 48 weeks after EOT. The research was conducted in accordance with the provisions of the World Medical Association Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Alexandria Faculty of Medicine (IRB No. 00007555). An informed consent was obtained from all subjects included in the study.

Clinical and laboratory as well as radiological data were evaluated at baseline. Conventional abdominal ultrasound was performed to assess the liver texture and the existence of cirrhosis, any focal hepatic lesion, splenic size, ascites and portal blood flow. The diagnosis of liver cirrhosis was based on clinical characteristics, laboratory tests and ultrasonographic findings and/or ARFI-LSM \geq 1.8 m/sec. Upper gastrointestinal endoscopy was done for cirrhotic patients for surveillance of esophageal varices. Baseline laboratory investigations included HCV antibodies, HBV surface antigen and core IgG antibodies, quantitative polymerase chain reaction (PCR) for HCV-RNA, complete blood count (CBC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum albumin, serum bilirubin, prothrombin time and international normalized ratio (INR) and serum creatinine. ARFI-LSM, FIB-4 and APRI scores were calculated for the included patients at baseline. Then repeated along with quantitative PCR for HCV-RNA, CBC, AST and ALT during the follow-up period at EOT, 12 weeks, 24 weeks, and 48 weeks after EOT.

Serum-based fibrosis scores

APRI and FIB-4 scores were used to assess the degree of liver fibrosis in the present study. FIB-4 was calculated as $[\text{Age (years)} \times \text{AST (IU/L)}] / [\text{platelet count (10}^9/\text{L)} \times \sqrt{\text{ALT (IU/L)}}]$ ^[21]. APRI was calculated as $[\text{AST (IU/L)} / \text{upper limit of normal (IU/L)}] \times [100 / \text{platelet count (10}^9/\text{L)}]$ ^[22].

ARFI technique and interpretation

ARFI elastography was done using Siemens Acuson S2000TM ultrasound system (Siemens AG, Erlangen, Germany) with the Virtual Touch Issue Quantification software and a 4C1 curved ultrasound probe was used to measure the liver stiffness of all patients included in the study. The procedure was performed by an experienced sonographer who was blinded to the clinical data throughout the study. After an overnight fast, each patient was placed in supine position with the arm in abduction and underwent B-mode scan through intercostal approach while holding the breath for a moment at the end of expiration to minimize breathing motion during the examination^[16]. A region of interest, of 10 mm length and 5 mm width at least 1-2 cm under the capsule and a maximum depth

of 8 cm in the right hepatic lobe parenchyma and free of any large blood vessels or biliary structures, was selected. Shear wave velocity was measured in meters per second (m/sec). Reliable ARFI-LSM was defined as the median of 10 valid measurements with a success rate $\geq 60\%$ and interquartile range to median value ratio less than 30%.

ARFI-LSM results were correlated to the different stages of liver fibrosis classified according to the METAVIR score. Cut-off values used for diagnosis and stratifying patients were as following: ARFI-LSM <1.34 m/sec for absent or mild fibrosis (F0 or F1), ARFI-LSM ≥ 1.34 - <1.55 m/sec for significant fibrosis (F2), ARFI-LSM ≥ 1.55 - <1.8 m/sec for severe fibrosis (F3), and ARFI-LSM ≥ 1.8 m/sec for cirrhosis (F4)^[12].

Statistical analysis

Data were fed to the computer and analyzed using the Statistical Package for Social Sciences (SPSS, version 20.0.) (Armonk, NY: IBM Corporation). Categorical data are presented as numbers and percentages. Continuous data are presented as mean \pm standard deviation (SD) or median (IQR). Variables between the different time-points of follow-up were compared using repeated-measures ANOVA and the Post Hoc Bonferroni's test for the parametric data, while the non-parametric data were compared using Friedman test and the Post Hoc Dunn's test. Percentage change in the distribution of fibrosis stages between the different time-points was analyzed by marginal homogeneity test. Baseline factors predicting the percent decrease of LSM at the end-point were analyzed using univariate and multivariate linear regression analyses. Statistical significance was assessed at $p < 0.05$. All calculated *P* values were two-tailed.

RESULTS

Between November 2018 and July 2020, a total of 174 treatment-naïve HCV-infected patients were included in the study. They all received a pan-genotypic sofosbuvir-based IFN-free DAAs regimen for 12 weeks. All patients achieved SVR12 and completed the follow-up visits till 48 weeks after the EOT. The baseline clinical, laboratory, radiological and endoscopic data of patients included in the study were listed in Table 1.

Serum aminotransferases and platelet count at the different time-points of the study

Serum aminotransferases showed continuous decline gradually overtime from the baseline till 12 weeks after the EOT. ALT declined further but this decline became significant only at 48 weeks after the EOT. AST showed a further significant decline between 12 and 24 weeks after the EOT then plateau. The median (IQR) of ALT declined significantly from 50.0 (37.0-78.0) at the baseline to 19.0 (15.0-25.0), 15.0 (13.0-20.0), 14.0 (12.0-18.0) and 14.0 (12.0-16.0) at the EOT, 12 weeks, 24 weeks and 48 weeks after the EOT respectively. Meanwhile, the median (IQR) of AST declined significantly from 43.50 (33.0-58.0) at the baseline to 21.0 (19.0-27.0), 19.0 (16.0-26.0), 21.0 (16.0-25.0) and 19.0 (15.0-22.0) at the EOT, 12 weeks, 24 weeks and 48 weeks after the EOT respectively. (Supplementary Table 1).

Platelet count increased significantly overtime from the baseline till 48 weeks after the EOT. The median (IQR) of platelet count increased from 198.5 (161.0-250.0) at the baseline to 212.5 (168.0-255.0), 214.0 (165.0-273.0), 220.0 (182.0-276.0) and 230.0 (180.0-290.0) at the EOT, 12 weeks, 24 weeks and 48 weeks after the EOT respectively (Supplementary Table 2).

Table 1 Distribution of the baseline clinical, laboratory, radiological and endoscopic data of patients included in the study.

Parameter	Median (IQR)/n (%)
Age (Years)	52 (43-58)
Gender (Male)	106 (60.9%)
Body mass index (Kg/m ²)	24 (23-27)
Hemoglobin level (g/dL)	14.8 (13.4 -15.8)
Platelet count (x10 ⁹ /L)	198.5 (161-250)
AST (U/L)	43.5 (33-58)
ALT (U/L)	50 (37-78)
Serum Albumin (g/dL)	4 (3.7-4.2)
Serum Bilirubin (mg/dL)	0.5 (0.3-0.70)
International Normalized Ratio	1.02 (1.0-1.13)
Serum-based fibrosis scores	
Baseline FIB-4	1.45 (0.87-2.43)
Baseline APRI	0.54 (0.36-1.16)
Baseline ARFI-LSM (m/sec)	1.49 (1.24-2.2)
Baseline fibrosis stage based on ARFI-LSM:	
F0-F1	55 (31.6%)
F2	42 (24.1%)
F3	22 (12.6%)
F4	55 (31.6%)
Endoscopy findings in cirrhotic patients:	
Absent gastro-esophageal varices	37/55 (67.3%)
Present gastro-esophageal varices	18/55 (32.7%)

Table 2 Estimated fibrosis stage on ARFI at the different time-points of the study for the patients subgroups.

ARFI	Baseline		End of therapy		SVR 12		SVR 24		SVR 48	
	No.	%	No.	%	No.	%	No.	%	No.	%
F0 - F1 (<1.34)	56	32.2	89	51.1	98	56.3	110	63.2	114	65.5
F2 (1.34 - <1.55)	41	23.6	23	13.2	25	14.4	16	9.2	14	8
F3 (1.55 - <1.8)	22	12.6	16	9.2	8	4.6	9	5.2	9	5.2
F4 (≥ 1.8)	55	31.6	46	26.4	43	24.7	39	22.4	37	21.3
<i>P</i> _{Baseline}			<0.001*		<0.001*		<0.001*		<0.001*	
<i>P</i> ₁					<0.001*		<0.001*		<0.001*	
Significance between periods							P ₂ <0.001*, P ₃ <0.001*, P ₄ =0.011*			

*P*₁: p-value for comparing between End of treatment and each other period; *P*₂: p-value for comparing between SVR12 and SVR24; *P*₃: p-value for comparing between SVR12 and SVR48; *P*₄: p-value for comparing between SVR24 and SVR48; *: Statistically significant at $P \leq 0.05$.

Serum-based fibrosis scores at the different time-points of the study

FIB-4 score demonstrated a continuous significant decline between the baseline and 48 weeks after the EOT, except the period between the EOT and 12 weeks after the EOT when there was no significant change observed. The median (IQR) of FIB-4 was 1.45 (0.87-2.43), 1.12 (0.80-1.81), 1.06 (0.76-1.83), 1.15 (0.70-1.77) and 1.10 (0.73-1.51) at the baseline, the EOT, 12 weeks, 24 weeks and 48 weeks after the EOT respectively (Figure 1) (Supplementary Table 3).

APRI values demonstrated a continuous significant decline between the baseline and 48 weeks after the EOT. The median (IQR) of APRI was 0.54 (0.36-1.16), 0.26 (0.20-0.43), 0.24 (0.19-0.39), 0.24 (0.18-0.38) and 0.23 (0.17-0.32) at the baseline, the EOT, 12 weeks, 24 weeks and 48 weeks after the EOT respectively (Figure 1) (Supplementary Table 3).

Table 3 Univariate and multivariate analysis for the variables affecting the decrease of ARFI-LSM at 48 weeks after the end of therapy in non-cirrhotic patient group (n =119).

% of decrease of ARFI-LSM	Univariate		#Multivariate	
	P	B (95%C.I)	P	B (95%C.I)
Male sex	0.065	-4.304 (-8.873 -0.264)		
Age (years)	0.153	-0.134 (-0.318 - 0.050)		
Body mass index (kg/m ²)	0.039*	-0.952 (-1.854 - -0.050)	0.101	0.573 (-0.114 - 1.260)
Hemoglobin level (g/ dl)	0.664	0.324 (-1.152 - 1.800)		
Platelet count (x10 ⁹ /L)	0.129	0.029 (-0.008 - 0.066)		
AST (U/L)	0.059	0.086 (-0.003 - 0.175)		
ALT (U/L)	0.145	0.038 (-0.013 - 0.089)		
APRI	0.41	2.168 (-3.029 - -3.029)		
FIB-4	0.102	-2.165 (-4.765 - 0.434)		
ARFI (m/ sec)	<0.001*	36.987 (30.399 -43.576)	<0.001*	37.902 (30.686 - 45.118)
Serum Bilirubin (mg/ dl)	0.496	2.858 (-5.427 - 11.144)		
Serum Albumin (g/ dl)	0.542	1.650 (-3.694 - 6.994)		
INR	0.002*	34.402 (12.419 - 56.386)	0.078	14.515 (-1.648 - 30.679)

C.I: Confidence interval; B: Unstandardized Coefficients;#: All variables with P < 0.05 were included in the multivariate analysis;*:Statistically significant at P ≤ 0.05.

Table 4 Univariate and multivariate analysis for the variables affecting the decrease of ARFI-LSM at 48 weeks after the end of therapy in cirrhotic patient group (n = 55).

% of decrease of ARFI-LSM	Univariate		#Multivariate	
	P	B (95%C.I)	P	B (95%C.I)
Male sex	0.107	-6.129 (-13.636 - 1.378)		
Age (years)	0.096	-0.297 (-0.649 - 0.054)		
Body mass index (kg/ m2)	0.106	-1.677 (-3.721 -0.368)		
Hemoglobin level (g/ dl)	<0.001*	3.770 (1.856 - 5.684)	<0.001*	-5.588 (-8.428 - -2.749)
Platelets (x 10 ⁹ /L)	<0.001*	0.079 (0.050 - 0.109)	<0.001*	0.138 (0.092 - 0.183)
AST (U/L)	0.291	-0.044 (-0.126 - 0.039)		
ALT (U/L)	0.445	0.015 (-0.023 - 0.052)		
APRI	0.014*	-3.476 (-6.207 - -0.745)	0.004*	4.901 (1.689 - 8.113)
FIB-4	<0.001*	-3.149 (-4.575 - -1.722)	0.12	1.660 (-0.448 - 3.768)
ARFI (m/s)	0.07	-6.761 (-14.088 - 0.565)		
Presence of varices by EGD	<0.001*	-10.802 (-15.955 - -5.650)	<0.001*	-24.318 (-32.571 - -16.066)
Serum Bilirubin (mg/ dl)	0.811	2.166 (-15.916 - 20.249)		
Serum Albumin (g/ dl)	<0.001*	18.928 (10.188 - 27.668)	<0.001*	16.772 (8.129 - 25.415)
INR	0.016*	-38.798 (-70.194 - -7.403)	0.086	-21.664 (-46.508 - 3.180)

C.I: Confidence interval; B: Unstandardized Coefficients; #: All variables with P < 0.05 were included in the multivariate analysis;*:Statistically significant at P ≤ 0.05

Changes of ARFI-LSM at the different time-points of the study

There was a continuous significant decline of ARFI-LSM from the baseline over the follow-up period. The median (IQR) of ARFI-LSM was 1.49 (1.24-2.20), 1.33 (1.20-1.93), 1.28 (1.15-1.76), 1.22 (1.08-1.68) and 1.20 (1.05-1.62) at the baseline, the EOT, 12 weeks, 24 weeks and 48 weeks after the EOT respectively (Figure 2) (Supplementary Table 4).

The distribution of fibrosis stage determined on the baseline ARFI-LSM values changed significantly at the EOT, 12 weeks, 24 weeks and 48 weeks after the EOT (Table 2) (Supplementary Figure 1).

Nearly 42.53 %of patients revealed fibrosis regression of at least 1 stage, meanwhile the fibrosis stage regressed at least 2 stages in 14.94% of patients by the end of follow-up period. The regression of fibrosis stage was more observed in patients with baseline F2-F3 fibrosis stage than patients with other fibrosis stages (Supplementary Table 4).

Predictors of fibrosis regression

In non-cirrhotic patients, the higher baseline ARFI-LSM, the lower

BMI and the higher INR showed significant impact on fibrosis regression in the univariate analysis. Excitingly, in the multivariate analysis, the higher baseline ARFI-LSM turned out to be an independent predictor of fibrosis regression at 48 weeks after the EOT (Table 3).

In cirrhotic patients, the higher platelet count, the higher hemoglobin concentration, the higher serum albumin level, the lower INR, the lower FIB-4 score, the lower APRI score and the absence of varices showed significant impact on fibrosis regression in the univariate analysis. However, in the multivariate analysis, the higher platelet count, the higher hemoglobin concentration, the higher serum albumin level, the lower APRI score and the absence of varices were collectively associated with fibrosis regression at 48 weeks after the EOT. Absent portal hypertension (represented as higher platelet count and absence of varices) and compensated liver disease (represented as higher serum albumin and platelet count and lower APRI score) in cirrhotic patients at baseline were associated with higher ARFI-LSM improvement at 48 weeks after the EOT (Table 4).

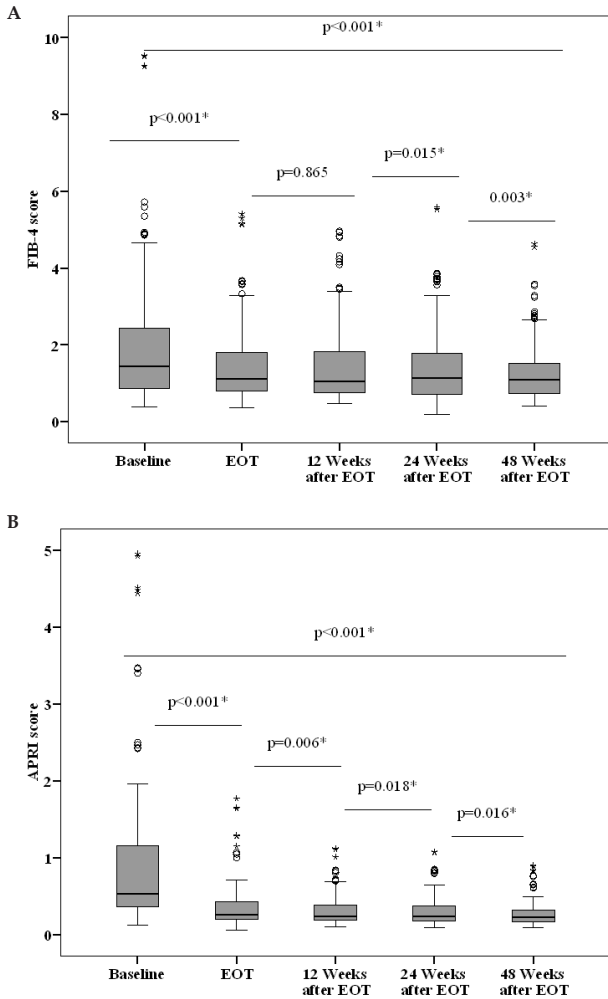


Figure 1 Serial changes in fibrosis scores at the different time-points of the study for the patient total sample.

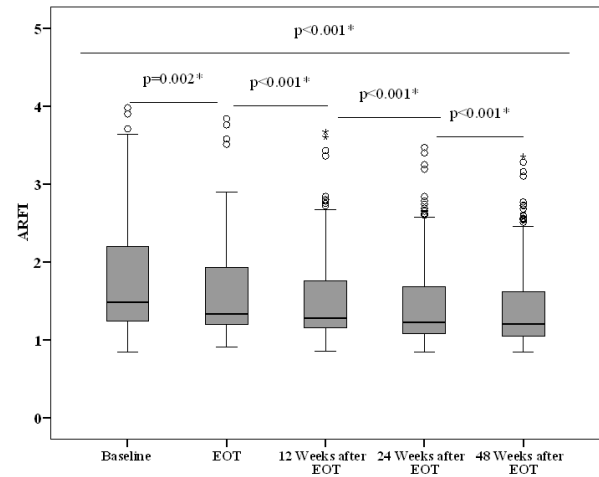
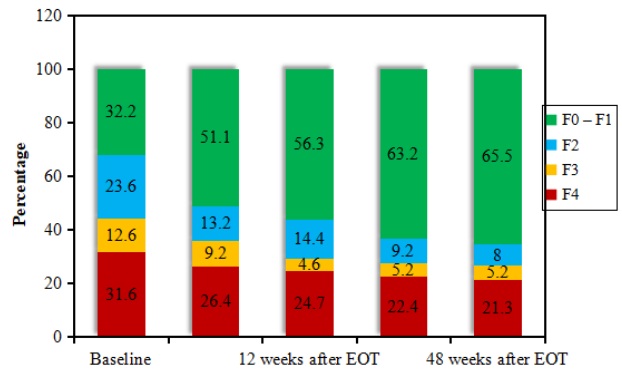


Figure 2 Serial changes in ARFI-LSM at the different time-points of the study for the patient total sample.



Supplementary Figure 1 Estimated fibrosis stage on ARFI-LSM at the different time-points of the study for the patient subgroups.

Supplementary Table 1 Statistical comparison of the serial changes of serum aminotransferases at the different time-points of the study for the patient total sample.

Parameter	Baseline	End of treatment	SVR12	SVR24	SVR48	Fr	p
AST							
Min. - Max.	13.0 - 174.0	11.0 - 109.0	11.0 - 51.0	12.0 - 45.0	12.0 - 37.0		
Mean ± SD.	53.37 ± 36.44	25.83 ± 15.12	21.61 ± 7.68	21.03 ± 6.87	19.58 ± 5.02	358.825*	<0.001*
Median (IQR)	43.50 (33.0 - 58.0)	21.0 (19.0 - 27.0)	19.0 (16.0 - 26.0)	21.0 (16.0 - 25.0)	19.0 (15.0 - 22.0)		
P Baseline		<0.001*	<0.001*	<0.001*	<0.001*		
P ₁			<0.001*	<0.001*	<0.001*		
Significance between periods			p ₂ =0.009*, p ₃ <0.001*, p ₄ =0.235				
ALT							
Min. - Max.	19.0 - 387.0	12.0 - 126.0	11.0 - 40.0	11.0 - 133.0	11.0 - 39.0		
Mean ± SD.	73.14 ± 70.29	24.17 ± 19.09	17.37 ± 6.75	17.12 ± 11.15	15.55 ± 5.11	427.760*	<0.001*
Median (IQR)	50.0 (37.0 - 78.0)	19.0 (15.0 - 25.0)	15.0 (13.0 - 20.0)	14.0 (12.0 - 18.0)	14.0 (12.0 - 16.0)		
P Baseline		<0.001*	<0.001*	<0.001*	<0.001*		
P ₁			<0.001*	<0.001*	<0.001*		
Significance between periods			P ₂ =0.060, P ₃ =0.040*, P ₄ =0.865				

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; IQR: Interquartile range; SD: Standard deviation; SVR: Sustained virological response; Fr: Friedman test; Significance between periods was done using Post Hoc Test (Dunn's test); P: p-value for comparing between the different periods; P Baseline: p-value for comparing between Baseline and each other periods; P₁: p-value for comparing between End of treatment and each other periods; P₂: p-value for comparing between SVR12 and SVR24; P₃: p-value for comparing between SVR12 and SVR48; P₄: p-value for comparing between SVR24 and SVR48; *: Statistically significant at P ≤ 0.05.

DISCUSSION/CONCLUSIONS

Treatment of HCV with the new DAAs is extremely effective, causing a very rapid control of viral replication, normalization of transaminases and disappearance of liver inflammation^[9]. Consequently, the virological and biochemical profile of these new treatments is definitely quite different from the previous IFN-based therapies, in which there is likely a slower and longer process taking place.

In the present study, all patients achieved undetectable HCV-RNA serum level at EOT. SVR was observed in 100% of patients at 12 weeks after EOT (SVR12) and continued till 48 weeks after EOT. In

the present study, serum aminotransferases decreased gradually from the baseline over the follow-up period. Okawa *et al.*^[23] reported that the mean ALT decreased significantly during the 12-week treatment period from 60.7 at baseline to 22.1 at EOT. Also, the mean ALT further decreased significantly until follow-up 12 weeks after EOT then there was no significant difference between follow-up 12 and 24 after EOT. Similar results were published by Tada *et al.*^[24] who found that ALT and AST levels decreased over time, and there were significant differences between baseline and EOT and between EOT and SVR24, however, there was no data of follow-up at SVR12. That was different in a study conducted by Hsu and colleagues^[25] who reported that the median of AST decline from 55 at baseline to

Supplementary Table 2 Statistical comparison of the serial changes in hematological parameters at the different time-points of the study for the patient total sample.

Complete blood picture	Baseline	End of treatment	SVR12	SVR24	SVR48	F	p
Hemoglobin							
Min. - Max.	10.50 - 17.0	10.60 - 16.80	9.70 - 16.90	10.50 - 16.80	11.0 - 16.50	12.492*	<0.001*
Mean ± SD.	14.43 ± 1.57	14.07 ± 1.41	14.10 ± 1.63	14.19 ± 1.57	14.15 ± 1.33		
Median (IQR)	14.80 (13.40 - 15.80)	14.0 (13.0 - 15.20)	14.10 (12.90 - 15.40)	14.30 (13.0 - 15.50)	14.0 (13.20 - 15.20)		
P Baseline		<0.001*	<0.001*	0.002*	<0.001*		
p1			1	0.411	1		
Significance between periods			p2=0.914,p3=1.000,p4=1.000				
Platelets							
Min. - Max.	27.0 - 404.0	69.0 - 445.0	77.0 - 376.0	78.0 - 392.0	84.0 - 395.0	19.741*	<0.001*
Mean ± SD.	205.2 ± 77.16	217.1 ± 72.42	217.0 ± 71.80	225.1 ± 73.46	229.7 ± 72.71		
Median (IQR)	198.5 (161.0 - 250.0)	212.5 (168.0 - 255.0)	214.0 (165.0 - 273.0)	220.0 (182.0 - 276.0)	230.0 (180.0 - 290.0)		
P Baseline		0.013*	0.003*	<0.001*	<0.001*		
p1			1	0.126	<0.001*		
Significance between periods			P2=0.017*,P3<0.001*,P4=0.273				
Leucocytes							
Min. - Max.	2.56 - 13.33	1.83 - 13.40	2.10 - 12.45	2.34 - 12.20	2.70 - 12.20	1.112	0.341
Mean ± SD.	6.33 ± 1.93	6.47 ± 1.91	6.49 ± 1.88	6.47 ± 1.70	6.40 ± 1.62		
Median (IQR)	6.23 (4.62 - 7.84)	6.10 (5.30 - 7.50)	6.44 (5.0 - 7.84)	6.50 (5.30 - 7.50)	6.50 (5.20 - 7.40)		

IQR: Interquartile range; SD: Standard deviation; SVR: Sustained virological response; F: F test (ANOVA) with repeated measures; Significance between periods was done using Post Hoc Test (adjusted Bonferroni's test); P: p-value for comparing between the different periods; P Baseline: p-value for comparing between Baseline and each other periods; P1: p-value for comparing between End of treatment and each other periods; P2: p-value for comparing between SVR12 and SVR24; P3: p-value for comparing between SVR12 and SVR48; P4: p-value for comparing between SVR24 and SVR48; *: Statistically significant at P ≤0.05.

Supplementary Table 3 Statistical comparison of the serial change in serum-based fibrosis scores at the different time-points of the study for the patient total sample.

Fibrosis scores	Baseline	End of treatment	SVR12	SVR24	SVR48	Fr	p
FIB-4 score							
Min. - Max.	0.39 - 9.53	0.37 - 5.41	0.46 - 4.96	0.18 - 5.58	0.40-4.63	142.272*	<0.001*
Mean ± SD.	2.02 ± 1.70	1.47 ± 0.99	1.46 ± 1.0	1.43 ± 1.01	1.29 ± 0.79		
Median (IQR)	1.45 (0.87-2.43)	1.12 (0.80-1.81)	1.06 (0.76-1.83)	1.15 (0.70-1.77)	1.10 (0.73-1.51)		
P Baseline		<0.001*	<0.001*	<0.001*	<0.001*		
p1			0.865	0.009*	<0.001*		
Significance between periods			p2=0.015*,p3<0.001*,p4=0.003*				
APRI score							
Min. - Max.	0.13 - 4.95	0.06 - 1.77	0.10 - 1.12	0.09 - 1.08	0.09 - 0.90	327.259*	<0.001*
Mean ± SD.	0.91 ± 0.95	0.38 ± 0.32	0.32 ± 0.23	0.30 ± 0.19	0.27 ± 0.16		
Median (IQR)	0.54 (0.36-1.16)	0.26 (0.20- 0.43)	0.24 (0.19- 0.39)	0.24 (0.18- 0.38)	0.23 (0.17- 0.32)		
P Baseline		<0.001*	<0.001*	<0.001*	<0.001*		
p1			0.006*	<0.001*	<0.001*		
Significance between periods			p2=0.018*,p3<0.001*,p4=0.016*				

APRI: Aspartate aminotransferase to platelet count ratio index; FIB-4: Fibrosis score 4; IQR: Interquartile range; SD: Standard deviation; SVR: Sustained virological response; Fr: Friedman test; Significance between periods was done using Post Hoc Test (Dunn's); P: p-value for comparing between the different periods; P Baseline: p-value for comparing between Baseline and each other periods; P1: p-value for comparing between End of treatment and each other periods; P2: p-value for comparing between SVR12 and SVR24; P3: p-value for comparing between SVR12 and SVR48; P4: p-value for comparing between SVR24 and SVR48; *: Statistically significant at P ≤0.05.

25, 25, and 27 at week 4, EOT, and SVR12 respectively. Also, the median of ALT decreased significantly from 66 at baseline to 22, 21, and 22 at week 4, EOT, and SVR12 respectively. Moreover, Pietsch *et al*^[26], observed a rapid reduction in the aminotransferases AST and ALT between baseline and SVR 24 which remained stable up to 96 weeks after EOT.

The present study showed that the mean platelet count increased significantly over the follow-up period from the baseline till 48 weeks after the EOT. In accordance with these findings, Pietsch *et al*^[26], found a significant increase in platelet count between baseline and SVR24 than remained stable till 94 weeks after EOT. Also, Tada *et al*^[24], reported that although the platelet counts did not significantly change from baseline to EOT, they increased significantly between EOT and SVR24. Moreover, in a retrospective study for a shorter

period, Elsharkawy *et al*^[27], reported a significant increase in platelet count in all subjects, but more in cirrhotic subgroup, between baseline and SVR12. On the other hand, Hsu *et al*^[25], observed that there was a significant increase in the median platelet count between week 2 and EOT in all subgroups of patients and at SVR12 in patients with thrombocytopenia while the degree of changes in platelet count was small. Karasuet *al*^[28], reported that hepatic necroinflammation and the liver fibrosis stage were inversely correlated to platelet count in chronic hepatitis B and CHC patients.

Various studies have reported liver fibrosis regression in most patients who achieved SVR to IFN therapy^[7,29,30]. In the new era of DAAs, higher rates of patients can achieve SVR in contrast with IFN-based therapy, but the impact of SVR on the reversal of fibrosis in these patients is still a main topic of research nowadays.

Supplementary Table 4 Statistical comparison of the serial change in ARFI-LSM at the different time-points of the study for the patient total sample and subgroups.

	ARFI					Fr	P
	Baseline	End of treatment	SVR12	SVR24	SVR48		
Patient total sample (n= 174)							
Min. - Max.	0.84 - 3.98	0.90 - 3.84	0.85 - 3.67	0.84 - 3.47	0.84 - 3.35	343.659	<0.001*
Mean ± SD.	1.74 ± 0.68	1.60 ± 0.62	1.53 ± 0.59	1.47 ± 0.59	1.43 ± 0.57		
Median (IQR)	1.49(1.24 -2.20)	1.33(1.20 - 1.93)	1.28(1.15 - 1.76)	1.22(1.08 - 1.68)	1.20(1.05 - 1.62)		
p Baseline		0.002*	<0.001*	<0.001*	<0.001*		
p1			<0.001*	<0.001*	<0.001*		
Significance between periods			p2<0.001*,p3<0.001*,p4<0.001*				
F0 - F1 (n= 55)							
Min. - Max.	0.84 - 1.31	0.90 - 1.31	0.85 - 1.33	0.84 - 1.31	0.84 - 1.28	45.939	<0.001*
Mean ± SD.	1.12 ± 0.13	1.13 ± 0.12	1.10 ± 0.11	1.06 ± 0.11	1.05 ± 0.09		
Median (IQR)	1.16(1.04 - 1.23)	1.14(1.05 - 1.24)	1.11(1.02 - 1.19)	1.06(0.99 - 1.16)	1.02(1.0 - 1.10)		
p Baseline		0.004*	0.567	0.097	0.002*		
p1			0.020*	<0.001*	<0.001*		
Significance between periods			p2=0.026*,p3<0.001*,p4=0.132				
F2(n= 42)							
Min. - Max.	1.34 - 1.52	1.14 - 1.59	1.07 - 1.69	0.92 - 1.60	0.96 - 1.54	124.699	<0.001*
Mean ± SD.	1.44 ± 0.06	1.33 ± 0.11	1.29 ± 0.14	1.20 ± 0.16	1.14 ± 0.14		
Median (IQR)	1.45(1.38 - 1.48)	1.31(1.24 - 1.24)	1.26(1.18 - 1.36)	1.19(1.10 - 1.26)	1.10(1.04 - 1.22)		
p Baseline		0.009*	<0.001*	<0.001*	<0.001*		
p1			0.157	<0.001*	<0.001*		
Significance between periods			p2=0.001*,p3<0.001*,p4=0.006*				
F3 (n= 22)							
Min. - Max.	1.55 - 1.79	1.08 - 1.93	1.07 - 1.81	1.12 - 1.77	1.05 - 1.75	74.218	<0.001*
Mean ± SD.	1.67 ± 0.07	1.51 ± 0.18	1.39 ± 0.15	1.32 ± 0.15	1.28 ± 0.14		
Median (IQR)	1.66(1.62 - 1.72)	1.56(1.50 - 1.60)	1.41(1.29 - 1.46)	1.29(1.22 - 1.37)	1.24(1.21 - 1.34)		
p Baseline		0.017*	<0.001*	<0.001*	<0.001*		
p1			0.182	<0.001*	<0.001*		
Significance between periods			p2=0.010*,p3<0.001*,p4=0.182				
F4 (n= 55)							
Min. - Max.	1.80 - 3.98	1.10 - 3.84	1.09 - 3.67	1.03 - 3.47	1.04 - 3.35	161.828	<0.001*
Mean ± SD.	2.61 ± 0.50	2.32 ± 0.60	2.22 ± 0.60	2.15 ± 0.59	2.09 ± 0.58		
Median (IQR)	2.53(2.26 - 2.88)	2.33(2.05 - 2.71)	2.22(1.82 - 2.59)	2.24(1.73 - 2.52)	2.19(1.67 - 2.48)		
p Baseline		<0.001*	<0.001*	<0.001*	<0.001*		
p1			0.005*	<0.001*	<0.001*		
Significance between periods			p2=0.205,p3<0.001*,p4=0.001*				

ARFI: Acoustic radiation force impulse; F: Fibrosis stage; LSM : liver stiffness measurement; IQR: Interquartile range; SD: Standard deviation; SVR: Sustained virological response; Fr: Friedman test; Significance between periods was done using Post Hoc Test (Dunn's test); P: p-value for comparing between the different periods; P Baseline: p-value for comparing between Baseline and each other periods; P1: p-value for comparing between End of treatment and each other periods; P2: p-value for comparing between SVR12 and SVR24; P3: p-value for comparing between SVR12 and SVR48; P4: p-value for comparing between SVR24 and SVR48; *: Statistically significant at P ≤0.05.

Since liver fibrosis persists even after the eradication of HCV, in spite of its gradual resolution after SVR is achieved, correct and continuous assessment of liver fibrosis stage is important to evaluate resolution of necroinflammation, fibrosis regression and liver-related end point conditions^[31,32]. Over the last years, several researches has been focused on the evaluation of liver elastography technique for the assessment of fibrosis regression after treatment of CHC^[32]. Previously, transient elastography (TE) reported long-term improvement in LSM associated with SVR after IFN-based treatments^[33]. In spite of the growing modes of shear wave-based elastography, ARFI still possesses promising and comparable validity and reliability for evaluating bidirectional fibrogenesis models^[19,34]. Furthermore, APRI and FIB-4 scores are commonly used non-invasive serum-based indices, and have been demonstrated to be reliable for predicting liver fibrosis in HCV-infected patients^[35].

In the present study, both FIB-4 and APRI scores demonstrated a continuous significant decline between the baseline and 48 weeks after the EOT, except the period between the EOT and 12 weeks after the EOT when there was no significant change observed in FIB-4 score. Similarly, Kohla *et al*^[36], demonstrated a significant reduction in the mean of FIB-4 and APRI values at EOT, week 24 and week 36 compared to baseline in CHC patients who received sofosbuvir-based DAAs. Similarly, Hsu and colleagues^[25] demonstrated that the decline in APRI and FIB-4 values occurred as early as 2 weeks after start of treatment and remained constant subsequently until SVR 24. Bachofner *et al*^[37], reported that the median FIB-4 and APRI values decreased between baseline and 18 months after EOT in all CHC patients who received DAAs.

In the present study, there was a continuous significant decline of ARFI-LSM from the baseline over the follow-up period, and the distribution of fibrosis stage determined on the baseline ARFI-LSM values changed significantly at the EOT, 12 weeks, 24 weeks and 48 weeks after the EOT. Both Hsu *et al*^[25], and Chen *et al*^[38] showed similar findings as regard the decrease of ARFI-LSM between baseline and 12 weeks after EOT, however, they assessed ARFI-LSM in total patients irrespective to fibrosis stage and only at 2 time-points till SVR12. The present study assessed ARFI-LSM at 5 time-points and in different fibrosis stages to give a better evaluation of the pattern of fibrosis regression in relation to time and fibrosis stage. Attia and colleagues^[39] observed continuous gradual decrease in ARFI-LSM in total HCV-infected patients and patients with cirrhosis, however, they found a non-significant change in patients without cirrhosis until 48 weeks after the EOT. In our study, ARFI-LSM showed continuous significant improvement at the EOT till 48 weeks after the EOT in all cirrhotic and non-cirrhotic patients. Moreover, we further subdivided the non-cirrhotic patients into different fibrosis stages to help better evaluation of regression in each fibrosis stage and we found that the degree of ARFI-LSM decline was more evident in patients with baseline F2 and F3 fibrosis stages compared to patients with baseline F0-F1 fibrosis stages.

LSM could be affected not only by the extent of liver fibrosis but also by necroinflammatory activity and intrahepatic portal pressure as well^[18]. Improvement of LSM that occurred at the end of therapy resulted in the question of whether these LSM readings reflect actual regression of fibrosis or rather an improvement in inflammation and associated interstitial edema. The influence of inflammation on the ARFI-LSM is still controversial, meanwhile numerous studies with TE have showed that LSM is affected by necroinflammatory activity. Fraquelli and colleagues^[40] demonstrated that fibrosis stage is likely overestimated by necroinflammatory activity and steatosis when measured by TE. On the contrary, Rizzo *et al*^[41] demonstrated that

ARFI-LSM was not associated with ALT level, body mass index or hepatic steatosis grade, whereas TE-LSM was significantly associated with ALT value by multivariate analysis. Bota *et al*^[42] reported that discordance of at least two stages of fibrosis between ARFI-LSM and histological assessment was associated with female sex, IQR $\geq 30\%$, elevated ALT and AST levels in a univariate analysis, although in a multivariate analysis, female sex and IQR $\geq 30\%$ were associated with this discordance. Moreover, Yoon *et al*^[18] showed that optimal cut-off values for ARFI-LSM decreased in patients with elevated ALT level from 1.13 m/sec to 1.09 m/sec for F2-F3, and from 1.98 m/sec to 1.81 m/sec for F4 in comparison to patients with normal ALT level.

In the present study, the early decrease in LSM in parallel to rapid normalization of aminotransferases till 12 weeks after the EOT is most likely a result of resolution of hepatic injury. But, it could not explain further reduction in ARFI-LSM after the EOT. In addition, the improvement in intrahepatic portal pressure may also explain the early significant reduction of LSM in a proportion of cirrhotic patients, an explanation which is shown in previous studies^[34,44]. The absence of portal hypertension at baseline in cirrhotic patients (represented as higher platelet count and absence of varices) was associated with higher ARFI-LSM improvement at 48 weeks after EOT by multivariate regression analysis in the current study. Accordingly, the LSM improvement at 48 weeks after EOT could not be attributed to improvement of intrahepatic portal pressure. It was expected that improvement of hepatic fibrosis was a slow process beginning in CHC patients who achieved SVR to DAAs therapy during the treatment and continued after that. So, the reduction of LSM at 48 weeks after EOT reflected mainly fibrosis regression. Nevertheless, to what extent improvements in LSM at 48 weeks after EOT mirror regression of liver fibrosis remains provisional as we had not obtained paired liver biopsies. However, this assumption might be supported by the finding that elevated serum aminotransferases as a marker of hepatic necroinflammation at baseline was not associated with the percentage of LSM improvement at 48 weeks after the EOT. The results of recent studies which paired liver biopsy with elastography could also support our explanation. Tachi *et al*^[45] demonstrated that higher inflammatory activity at baseline was related to the improvement in ARFI-LSM with an odd ratio of 1.940 at the EOT, whereas significant fibrosis at baseline was linked to the improvement in ARFI-LSM with an odd ratio of 2.617 at 24 weeks after the EOT.

In conclusion, DAAs therapy was associated with continuous reduction of ARFI-LSM after HCV eradication due to resolved hepatic necroinflammation and fibrosis regression. The presence of significant portal hypertension at baseline seemed to be the point of no return in fibrosis evolution depicted as liver stiffness. ARFI is a feasible, easily applicable and reproducible non-invasive method for pre- and post-treatment assessment of hepatic fibrosis.

Monitoring the evolution of HCV disease after viral cure is extremely important. ARFI is not the most frequently used non-invasive method for monitoring fibrosis evolution, but even so, it is extremely important to obtain data that support and validate the information given by this technique. In order to correct for the limitations of the current study, it may be recommended to use paired liver biopsies for histological confirmation or examine the patients with TE for comparison with ARFI data. It is imperative to explore the mechanistic of fibrosis generation and how that fibrosis could reverse after treatment of the causative injury. The fact of fibrosis regression after antiviral therapy and the validity of ARFI to estimate the stage of hepatic fibrosis should be extensively studied in

prospective longitudinal studies of large-scale population and longer follow-up period with inclusion of patients with etiologies of chronic liver disease other than HCV infection.

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Data Availability Statement

Datasets on which the conclusion of the paper rely are available to editors, reviewers and readers without unnecessary restrictions wherever possible.

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