Morphometry Confirms Fibrosis Regression From Sustained Virologic Response to Direct-Acting Antivirals for Hepatitis C

Jason J. Pan,[1] Fei Bao,[2] Emma Du,[2] Chase Skillin,[1] Catherine T. Frenette,[1,3] Jill Waalen,[4] Lakshmi Alaparthi,[5] Zachary D. Goodman,[5] and Paul J. Pockros[1,4,6]

Sustained virologic response (SVR) after direct-acting antiviral (DAA) therapy for chronic hepatitis C results in significant decreases in liver stiffness measured by transient elastography (TE). The aim of this study was to clarify if TE can guide post-SVR management in patients with advanced fibrosis or cirrhosis prior to treatment as current guidelines are unclear on the role of TE after SVR. In total, 84 patients with hepatitis C virus and advanced fibrosis or cirrhosis and from a single center underwent DAA treatment and achieved SVR. Overall, 62% had improved liver stiffness that was consistent with regression of at least one stage of fibrosis. In the cirrhosis group, 48% showed fibrosis regression by at least two stages by TE (<9.5 kPa). In the F3 fibrosis group, 39% regressed by at least two stages (<7 kPa). The median time from SVR to regression by TE was 1 year. Fifteen patients with liver biopsies prior to SVR underwent a biopsy after SVR; 13 of these patients had improved liver stiffness (to <9.5 kPa). The post-SVR liver biopsies of only 4 patients showed F1-F2 while 11 patients showed F3-F4; however, morphometry of the first 11 biopsied patients revealed that 10 patients had an average 46% decrease in collagen content. *Conclusion*: **This is the first DAA study that also has paired liver biopsies showing fibrosis regression. After SVR is achieved, improvements in liver stiffness measured by TE are seen in a majority of patients with advanced fibrosis/cirrhosis within 2 years. TE improvements are overstated when compared to histologic staging but confirmed with morphometric analysis. It is unclear whether TE following SVR can reliably predict when patients no longer require advanced fibrosis/cirrhosis monitoring after SVR. (*Hepatology Communications* 2018;2:1320-1330)**

Vibration-controlled transient elastography (TE) has been shown to accurately detect advanced fibrosis and cirrhosis in patients with chronic hepatitis C virus (HCV) infections.[1] However, the use of TE is limited by active inflammation and/or edema of the liver, which can cause overestimation of the degree of fibrosis.[2] Other factors known to confound TE include obesity, waist circumference, ascites, hepatic congestion, extrahepatic cholestasis, and eating within 4 hours of the exam.[3] Despite these limitations, TE offers a simple and rapid bedside assessment of fibrosis for many patients.

Multiple reports have demonstrated that novel direct-acting antiviral (DAA) therapies for HCV result in dramatic improvement in liver stiffness measured by TE in patients with sustained virologic response (SVR).[4-7] Improvements in liver stiffness can be observed as early as the end of treatment and continue

Abbreviations: ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; AST, aspartate aminotransferase; DAA, direct-acting antiviral; FIB-4, fibrosis 4; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; SVR, sustained virologic response; TE, transient elastography.

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even 12 months after therapy completion, with liver stiffness improvements ranging from –2 to –10 kPa.[4] This decrease in liver stiffness is associated with lowering of liver enzymes, improvements in fibrosis-4 (FIB-4) and aspartate aminotransferase-to-platelet ratio index (APRI) fibrosis scores, and an increase in platelets.[6,7] A recent study in Georgia[8] demonstrated reversal of TE scores in 304 patients with advanced fibrosis or cirrhosis following SVR at a similar rate to that reported by Crissien et al.[9]

However, a correlation between fibrosis regression by TE and histology is lacking in the DAA-era literature. These critical data are needed for physicians who are monitoring patients with advanced fibrosis or cirrhosis after SVR following DAA treatment. Previous evidence has shown a lower risk of hepatocellular carcinoma (HCC) and other liver-related complications with fibrosis regression following interferon-based regimens,[10] but the regression of fibrosis seems to be a very slow process.[11] Further, some patients did not regress after SVR and some even worsened, with an increased risk for HCC.[12] This potential complication has created an abundance of caution from clinicians and updates to guidance documents recommending indefinite screening for HCC in patients who achieved SVR who had advanced fibrosis or cirrhosis.[13] The guidelines issued by the European Association for the Study of the Liver and the Asociación Latinoamericana para el Estudio del Hígado have indicated that the routine use of noninvasive tests during treatment or after SVR in patients without cirrhosis does not add to clinical disease management. The guidelines also indicate that the routine use of noninvasive tests after SVR in patients with HCV cirrhosis has a high false-negative rate and cannot be used to determine which patients no longer need HCC screening or for the diagnosis of cirrhosis reversal. They further indicate that the routine use of noninvasive tests after SVR has not established thresholds that predict low risk of liver-related events.[14] Despite these recommendations, TE is being performed routinely in the community after SVR so demonstrating the correlation between biopsy and TE has important implications for clinical practice.

In May 2017, the American Gastroenterological Association published guidelines recommending a TE cutoff of <9.5 kPa to rule out advanced liver fibrosis in patients with chronic HCV without cirrhosis who achieved SVR after antiviral therapy.[15] The cutoff of <9.5 kPa would be expected to misclassify 1% of patients as not having advanced fibrosis in a low-risk population and 7% in a high-risk population, which would include patients with liver stiffness >12.5 kPa before therapy or other risk factors for chronic liver disease. This conditional recommendation was rated to have low-quality evidence per the Grading of Recommendations Assessment, Development, and Evaluation framework as actual comparative post-SVR data have not been available.[15]

Health care providers and patients thus have no guidance as to when to discontinue monitoring for HCC and complications from portal hypertension. The most recent estimate of the number of patients that

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ARTICLE INFORMATION:

From the [1]Division of Gastroenterology/Hepatology, Scripps Clinic, La Jolla, CA; [2]Department of Pathology, La Jolla, CA; [3]Division of Organ Transplantation, Scripps Green Hospital and Scripps Clinic, La Jolla, CA; [4]The Scripps Research Institute, La Jolla, CA; [5]Center for Liver Disease, Inova Medical Campus, Falls Church, VA; [6]The Scripps Translational Science Institute, La Jolla, CA.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Paul Pockros, M.D.
10666 North Torrey Pines Road
La Jolla, CA 92037

E-mail: pockros.paul@scrippshealth.org
Tel: +1-858-554-5579

fall into this category is 25%-37% of all HCV-infected individuals in the United States.[16] Therefore, this is not a trivial concern as it has significant clinical impact on 200,000 or more successfully cured patients.

The aim of this study was to clarify if TE can guide post-SVR management in patients with advanced fibrosis or cirrhosis prior to treatment as current guidelines are unclear on the role of TE after SVR.

PATIENTS AND METHODS

A retrospective review of patient referrals between 2010 and 2015 to the hepatology department at Scripps Clinic identified patients for this study. Patients included were at least 18 years old with chronic HCV with fibrosis of at least F3 staging who achieved SVR at 12 and 24 weeks posttreatment. Exclusion criteria included liver transplant prior to therapy, SVR without DAA therapy, failure to achieve SVR, age less than 18 years old, liver fibrosis less than F3, and additional causes of liver fibrosis other than HCV. Written informed consent was obtained per the protocol of the hospital's institutional review board, which reviewed and approved this study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Patient's baseline fibrosis prior to DAA therapy was assessed by liver biopsy, TE, and/or clinical signs.

Patients with clinical evidence of portal hypertension (varices, portal hypertensive gastropathy, ascites, and/or hepatic encephalopathy) or baseline TE greater than 12 kPa were categorized as having cirrhosis; otherwise, patients were placed in the advanced fibrosis (F3) group (Table 1). Patients underwent DAA therapy between January 2010 and October 2015. In patients who achieved SVR at 12 and 24 weeks, fasting TE measurements consisting of 10 measurements with an interquartile range of <25% were collected at 6- to 12-month intervals for up to 5.5 years. TE measurements began in October 2013 and were performed at the same institution using the same FibroScan 502 Touch machine by one of four operators. Fibrosis staging cutoffs for TE were as follows: F0-F1, <7 kPa; F2, 7-9.4 kPa; F3, 9.5-11.9 kPa; F4, >12 kPa; these were derived from values used in meta-analyses and systematic reviews.[17,18] HCC was screened regularly with ultrasound, computed tomography, and/or magnetic resonance imaging as needed.

Fourteen patients who had improvement in their fibrosis by at least one stage by TE had additional informed consent discussions regarding obtaining liver biopsies to confirm improvement. One patient who showed unchanging liver stiffness also wished to pursue liver biopsy. Liver biopsies were processed and evaluated using standard techniques; sinusoidal fibrosis was additionally evaluated. Previous biopsies were requested and compared by the same pathologist whenever possible to assess fibrosis regression. The biopsies were all read independently by two expert pathologists (F.B., E.D.) who were blinded to any clinical information and to the timing with respect to antiviral treatment. Discrepancies were solved by consensus reading.

Liver biopsy fibrosis stage was assessed according to the Batts-Ludwig scoring system as follows[19] : F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with septal fibrosis; F3, bridging fibrosis with architectural distortion; F4, cirrhosis. Similarly, hepatic inflammation was evaluated according to both the METAVIR and Batts-Ludwig grading systems.[19,20] The 11 biopsies used for morphometric measurements were read by a third pathologist (Z.D.G.) using the Batts-Ludwig and Ishak scoring systems.

For quantification of fibrosis, sections were stained with picrosirius red, which binds stoichometrically to collagen. A digitized image of each entire stained section was acquired using an Aperio/Leica Scanscope XT scanner at 20× magnification. The image analysis process also included a manual editing step to determine the total stained area of the section and to eliminate image artifacts. An area quantification algorithm (Indica Labs, Inc.) was used to quantify the number of red-stained pixels of the collagen fibers. Accuracy of classification was confirmed by visual inspection, and results were expressed as a fraction of the total pixels positive for picrosirius red (personal communication from Z.D.G.).

STATISTICAL METHODS

Characteristics between the cirrhosis and advanced fibrosis groups were summarized (Table 1), and differences were tested by two-sample *t* tests for continuous variables and Pearson χ^2 tests for categorical variables. Descriptive statistics were calculated for the primary outcome of changes in liver stiffness and improvements in fibrosis by biopsy. Changes in liver stiffness and liver chemistries following SVR were compared to pre-SVR levels by paired *t* tests. Time to improvement, defined as the time from SVR to one stage of fibrosis regression on TE, was assessed in both the cirrhosis and advanced fibrosis groups using

TABLE 1. BASELINE CHARACTERISTICS PRIOR TO SVR

	Overall (n = 84)	Cirrhosis (n = 56)	F3 (n = 28)	*P* value
Mean age (years)	60	60 (40-78)	59 (51-74)	0.46
Mean BMI (kg/m^2)	28	28 (19-40, n = 56)	27 (20-36, n = 25)	0.46
Male	53	33	20	0.26
Non-Hispanic white	73	48	25	0.65
Hispanic	8	6	2	0.60
Asian	3	2	1	1
Genotype 1	77	52	25	0.58
2	3	3	0	0.2
3	1	0	1	0.15
4	0	0	0	
5	0	0	0	
6	0	0	0	
Unknown	3	1	2	0.21
Treatment naive	36	21	15	0.16
Treatment experienced	48	35	13	
Pre-SVR biopsy				
Cirrhosis	20	20	0	<0.001
F3-F4	39	11	28	<0.001
F2	4	4	0	0.15
TE predicted stage				
Cirrhosis (≥12 kPa)	27	27	0	<0.001
F3-F4 (9.5-11.9 kPa)	1	0	1	0.15
Clinical portal hypertension	32	32	0	<0.001
PHG/varices	31	31	0	<0.001
Ascites*	3	3	0	0.21
Hepatic encephalopathy*	3	3	0	0.21
ALT (n = 80)	122	122 (30-664)	123 (22-439)	0.97
AST (n = 78)	87	90 (20-303)	81 (14-337)	0.58
Platelets (n = 80)	151	133 (34-362)	194 (95-290)	<0.001
INR (n = 78)	1.1	1.1 (0.9-2.9)	1.0 (0.9-1.3)	0.12
Albumin (n = 79)	3.9	3.8 (3.2-4.6)	4.0 (3.3-4.8)	0.16
FIB-4 score (n = 78)	4.1	4.7 (0.9-19.7, n = 55)	2.8 (0.5-8.5, n = 23)	<0.01

Abbreviations: BMI, body mass index; INR, international normalized ratio; PHG, portal hypertensive gastropathy.
*3 patients with compensated cirrhosis developed ascites documented by imaging and hepatic encephalopathy while being treated with telaprevir/pegylated interferon/ribavirin.

Kaplan-Meier curves and compared using the log rank test. Statistical significance was defined as $P < 0.05$.

PRIMARY AND SECONDARY OUTCOMES

The primary outcomes were liver stiffness in kPa and subsequently fibrosis staging predicted by TE after SVR was achieved compared to liver biopsy fibrosis staging and morphometry in a subset of patients. Secondary outcomes included the analysis of FIB-4 and APRI before and after therapy and the prevalence of HCC in this post-SVR population.

Results

PATIENT BASELINE DEMOGRAPHICS

Initially, 224 patients from a single center were eligible for the study. Three patients declined to participate and 28 patients were excluded for the following reasons: 1 patient had a liver transplant, 10 had multiple etiologies for their liver disease, and 17 were treated without DAA therapy. Of those patients who were treated in 2015 and later, 109 are still in follow-up, pending final analysis.

We included 84 patients in this analysis (Table 1). The mean age was 60 years old and mean body mass index was 28 kg/m^2 (SD, 4.6 kg/m^2). The study population was non-Hispanic white (87%), Hispanic (10%), or Asian (3%). The majority of patients were genotype 1A or 1B. We found 57% of the patients to be treatment experienced, and most had previously received interferon and ribavirin. The majority (55%) of patients were treated with sofosbuvir-based regimens (Supporting Table S1), 35% of patients received telaprevir-based therapy, and 10% were in DAA clinical trials, which included NS3/4A protease inhibitors and/or NS5A/B inhibitors, sometimes in combination with ribavirin and/or interferon.

Fifty-six patients were in the cirrhosis group; 28 patients were in the F3 group (Table 1), and 4 of these patients had pre-SVR biopsies that revealed F2 or F3 fibrosis and were classified as having cirrhosis due to a recent TE with liver stiffness >12 kPa, clinical evidence of portal hypertension, or both. The cirrhosis group had significantly higher mean FIB-4 scores ($P < 0.01$) and lower platelets ($P < 0.001$) than the F3 group. The cirrhosis group also had a lower mean albumin level, but this was not statistically significant. None of the patients had decompensated cirrhosis at the time of enrollment. Three patients developed ascites and hepatic encephalopathy during their antiviral therapy with telaprevir, pegylated interferon, and ribavirin; however, all 3 had compensated cirrhosis at the time this study began, and resolution of ascites was documented by ultrasound or computed tomography prior to enrollment.

LIVER STIFFNESS REGRESSION

Among the 56 patients who had cirrhosis prior to SVR, 23 patients (41%) continued to have cirrhosis (≥12 kPa) predicted by TE following SVR (Table 2). Thirty-three patients (59%) had improved by at least one stage (<12 kPa) and 27 patients (48%) had improved by at least two predicted stages of fibrosis (<9.5 kPa) after SVR. The median time to improvement was 1 year (95% confidence interval, 1.2 to 1.9 years; Figs. 1 and 2). Patients with pre-existing esophageal varices and/or who had or developed diabetes mellitus type 2 were less likely to improve their TE scores.

Among the 28 patients who had F3 fibrosis prior to SVR, 9 patients (32%) had either worsened or unchanged stiffness (≥9.5 kPa) by TE (Table 2) following SVR, 7 patients progressed to cirrhosis (≥12 kPa), and 2 patients remained in F3 (9.5 to 11.9 kPa). Improved liver stiffness by at least one stage of fibrosis (<9.5 kPa) was experienced by 19 patients (68%), and 11 patients (39%) had improved liver stiffness by at least two stages of fibrosis (<7 kPa). Median time to improvement was 1.5 years (95% confidence interval, 1.1 to 1.8 years; Figs. 1 and 2). Overall, among the 84 patients with advanced fibrosis or cirrhosis, 52 patients (62%) had decreased liver stiffness.

LABORATORY RESULTS

Statistically significant changes occurred in liver chemistries and FIB-4 scores between pre-SVR and the

TABLE 2. RESULTS STATUS AFTER SVR

	Overall (n = 84)	Cirrhosis (n = 56)	F3 (n = 28)
Post-SVR TE			
F4 (≥12 kPa)	30	23	7
F3 (9.5-11.9 kPa)	8	6	2
F2 (7-9.4 kPa)	20	12	8
F0-F1 (<7 kPa)	26	15	11
Mean follow-up time with TE (years)	2.0	1.9	2.3
ALT	35	35 (13-171)	34 (12-140)
AST	28	28 (13-87)	27 (14-85)
Platelets	173	153 (23-366)	215 (102-450)
INR	1.1	1.2 (0.9-4)	1.0 (1-1.1)
Albumin	4.1	4.1 (3.1-4.7)	4.2 (3.6-5)
FIB-4	2.22	2.60 (0.5-13.5)	1.43 (0.6-3.3)
HCC	4	2	2

Abbreviation: INR, international normalized ratio.

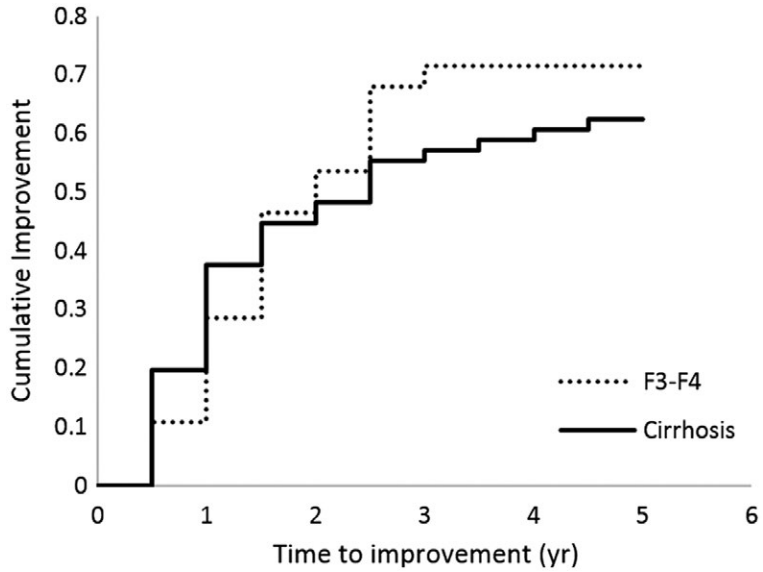

FIG. 1. Time to improvement from SVR by one stage of fibrosis estimated by TE. Median time to improvement was 1.5 years for F3-F4 (95% CI, 1.1-1.8 years) and 1 year for the cirrhosis group (95% CI, 1.2-1.9 years). Log-rank statistic $P = 0.08$. Abbreviation: CI, confidence interval.

most recent post-SVR values (Table 2). In the cirrhosis group, mean alanine aminotransferase (ALT) changed from 122 to 35 ($P < 0.001$), aspartate aminotransferase (AST) from 90 to 28 ($P < 0.001$), platelets from 133 to 153 ($P < 0.001$), and albumin from 3.8 to 4.1 ($P < 0.001$). The mean FIB-4 score among patients with

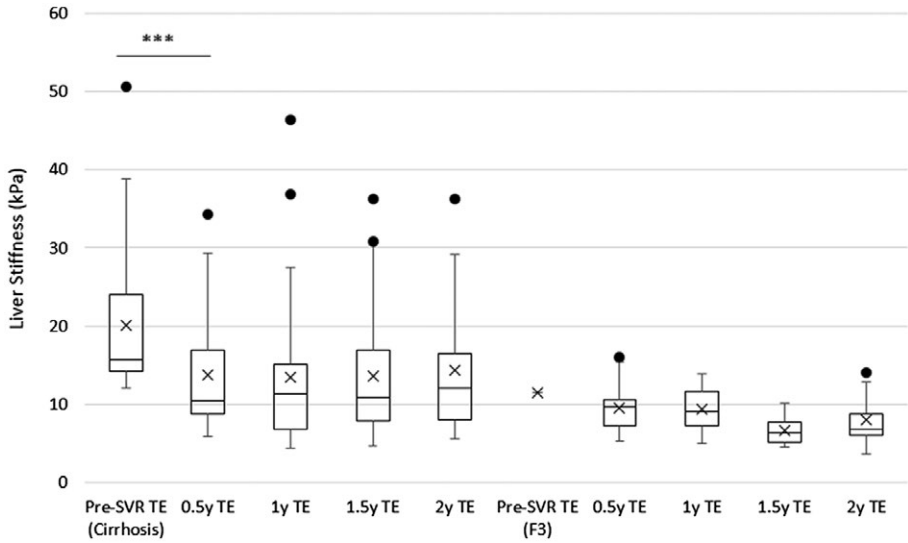

FIG. 2. Pre-SVR and post-SVR TE values (kPa) for the cirrhosis group and F3 group. Box plots of pre-SVR and post-SVR TE values from 0.5 to 2 years after SVR. The box consists of first to third quartile values, with the median line marked; whiskers extend to the farthest data point within 1.5 of the interquartile range. Outliers outside of 1.5 of the interquartile range are marked with a circle, and the mean of each group is marked by an X. The three asterisks denote statistical significance with $P < 0.001$ for a paired t test comparison between the pre-SVR and 0.5 years after the post-SVR samples. There was only one baseline TE reading in the F3 group so statistical testing was unable to be performed. Abbreviation: y, year.

cirrhosis decreased from 4.7 to 2.6 ($P < 0.001$). In the F3 group, mean ALT changed from 114 to 34 ($P < 0.001$), AST from 81 to 27 ($P < 0.001$), platelets from 194 to 215 ($P = 0.06$), and albumin from 4 to 4.2 ($P = 0.12$). The FIB-4 score in the advanced fibrosis group decreased from 2.8 to 1.4 ($P < 0.01$).

HCC

Of the 84 patients, 4 (4.8%) were found to have HCC. Prior to DAA therapy, 2 patients had cirrhosis and 2 had F3 fibrosis. In the cirrhosis group, 1 patient had HCC prior to starting DAA therapy and the other was diagnosed 3 years after treatment with telaprevir, pegylated interferon, and ribavirin. In the F3 group, 1 patient was diagnosed with HCC 6 months after treatment with telaprevir, pegylated interferon, and ribavirin and was treated with wedge resection, which revealed cirrhotic liver parenchyma surrounding HCC. The other was diagnosed 2 years after treatment with ledipasvir and sofosbuvir and was found to have cirrhosis at the time of partial liver lobectomy.

LIVER BIOPSY STATUS POST-SVR

Fifteen patients underwent a repeat liver biopsy after achieving SVR, and all biopsies were deemed to have adequate sample size for analysis by one (n = 4) or two (n = 11) pathologists. Prior to DAA therapy, 8 patients had cirrhosis and 7 patients had F3 fibrosis (Table 3). In the cirrhosis group, the highest level of fibrosis observed on repeat biopsy after SVR was F3 and the lowest was F0-F1. These biopsied patients with cirrhosis were predicted per American Gastroenterological Association guidelines by TE to have at least one stage improvement (<12.5 kPa). Six patients with cirrhosis were predicted by TE to have F0-F1 fibrosis (<7 kPa), and 2 patients with cirrhosis were predicted to have F2-F3 fibrosis (7-11.9 kPa) after SVR. Their liver biopsies revealed that 2 patients had F1-F2 fibrosis and 6 patients had features of F3-F4 fibrosis. In this group with dramatic improvements on TE, the pathologist also reported that 3 patients had reduced sinusoidal fibrosis compared to their prior biopsy and 2 patients did not have any sinusoidal fibrosis in their current biopsy (but their prior biopsy was not available to determine sinusoidal fibrosis regression). Among the 7 F3 patients, 6 patients were predicted by TE to have F0-F1 fibrosis (<7 kPa) but only two post-SVR liver biopsies were noted to be F1-F2 (Table 3). The other five post-SVR biopsies contained features of F3 fibrosis. A reduction in sinusoidal fibrosis was appreciated in 3 patients in the F3 group.

Improvements in inflammation were experienced by 11 of the 15 post-SVR biopsies with a post-SVR Batts-Ludwig activity grade of 1 from a pre-SVR grade of 2-3. One patient with cirrhosis had a post-SVR activity grade of 2 from grade 3, and 3 patients did not show any change. Two patients had grade 1 activity in both pretreatment and posttreatment biopsies, and another had grade 2 activity in both biopsies.

In subgroup analysis, 3 patients with cirrhosis and 3 F3 patients were treated with interferon (Supporting Table S2). The TE of all 6 patients was consistent with F0-F1. The post-SVR biopsy of the patients with cirrhosis revealed one F2 and two F3. The post-SVR biopsy of the F3 patients revealed two F1-F2 and one F2-3.

In the cirrhosis group of the repeat biopsy subset, APRI scores decreased from 2.5 to 0.4 ($P = 0.04$) and FIB-4 scores from 4.29 to 1.95 ($P = 0.02$). In the F3 group, APRI scores decreased from 0.9 to 0.3 ($P = 0.07$) and FIB-4 scores from 2.58 to 1.21 ($P = 0.14$). Of the 15 patients with cirrhosis and advanced fibrosis, 13 had significant improvements in their liver stiffness by TE (<9.5 kPa) after SVR was achieved. The liver biopsies of 4 patients had a staging of F1-F2; the other 9 patients had liver biopsies with features of F3 or F4 fibrosis (Fig. 3).

MORPHOMETRIC ANALYSIS OF LIVER BIOPSIES

Morphometric analysis was done on the first 11 of the 15 patients who underwent post-SVR biopsy. Of these 11 patients, 10 had a decline in collagen by an average of 46% over varying time intervals (Fig. 4). The 1 subject with an increase in collagen had the lowest amount of baseline collagen and had demonstrated improvements in liver biopsy (F3 to F1-F2) and TE (post-SVR TE <9.5 kPa). The mean percentage of collagen decreased from 7.1% to 3.8% ($P < 0.01$).

Discussion

TE with a cutoff of 9.5 kPa and 12.5 kPa has been recommended to stratify patients into advanced fibrosis and cirrhosis, respectively, including patients who have been treated with DAAs.[15] In the present study, 13 of the 15 patients with advanced fibrosis or cirrhosis

TABLE 3. REPEAT BIOPSY DATA OF SVR PATIENT SUBSET

	Overall (n = 15)	Cirrhosis (n = 8)	F3 (n = 7)
Mean age (years)	61	60	61
Male	9	4	5
Mean BMI (kg/m^2)	27	27	27
Non-Hispanic white	13	7	6
Hispanic white	1	0	1
Asian	1	1	0
Genotype 1A	12	8	4
Genotype 1B	2	0	2
Unknown	1	0	1
Treatment naive	9	3	6
Treatment experienced	6	5	1
Initial biopsy			
Cirrhosis	3	3	0
F3-F4	12	5	7
Pre-SVR TE predicted			
F4 (≥12 kPa)	3	3	0
F3 (9.5-11.9 kPa)	1	0	1
Varices/PHG	4	4	0
HE	0	0	0
Ascites	0	0	0
HCC	0	0	0
Mean post-SVR TE	6.7 kPa	6.9 kPa	6.4 kPa
F4 (≥12 kPa)	1	0	1
F3 (9.5-11.9 kPa)	1	1	0
F2 (7-9.4 kPa)	1	1	0
F0-F1 (<7 kPa)	12	6	6
Post-SVR biopsy			
Cirrhosis	0	0	0
F3-F4	3	1	2
F3	2	2	0
F2-3	6	3	3
F1-F2	4	2	2
Pre-SVR			
APRI	1.8	2.5	0.9
FIB-4	3.49	4.29	2.58
Post-SVR			
APRI	0.3	0.4	0.3
FIB-4	1.61	1.95	1.21
Mean time from SVR to biopsy (years)	3	2.3	3.9

Abbreviations: BMI, body mass index; HE, hepatic encephalopathy; PHG, portal hypertensive gastropathy.

who underwent a repeat liver biopsy had liver stiffness <9.5 kPa at the time of their repeat biopsy. However, the biopsies of only 4 patients (31%) had fibrosis without F3-F4 features (Supporting Figure S1). In the 2 patients with liver stiffness >9.5 kPa, both continued to have F3 fibrosis, suggesting that TE remains an effective tool for confirming advanced fibrosis but may not be specific enough to confirm resolution of advanced fibrosis.

A previous histologic study using morphometric analysis in paired liver biopsies from 37 patients with cirrhosis due to HCV demonstrated reversal of fibrosis and morphometry scores following SVR after interferon-based therapy.[21] The regression of area of fibrosis measurement in this previous study was similar to that reported in the present study. These findings suggest that, similar to patients treated with interferon-based regimens, the degree of liver fibrosis regression from

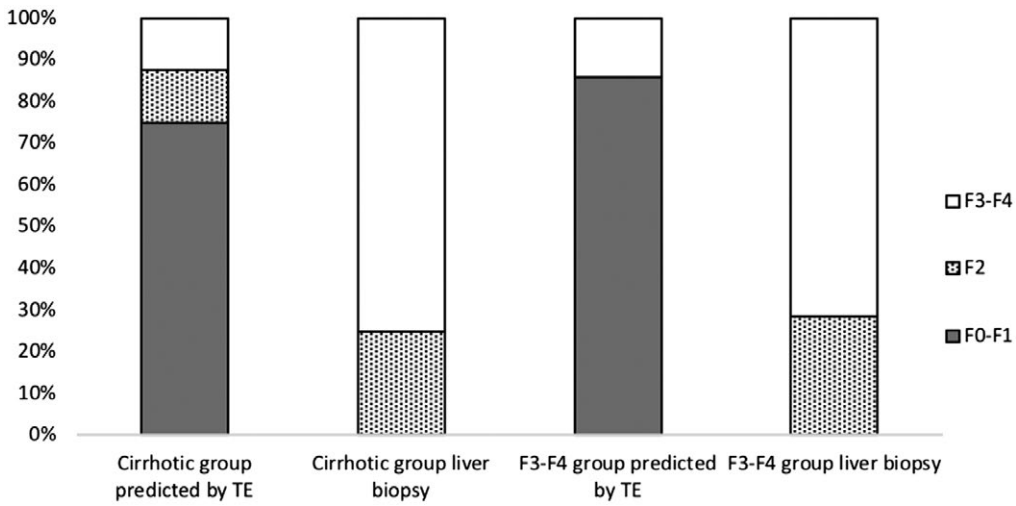

FIG. 3. Post-SVR fibrosis stage predicted by TE compared to liver biopsy.

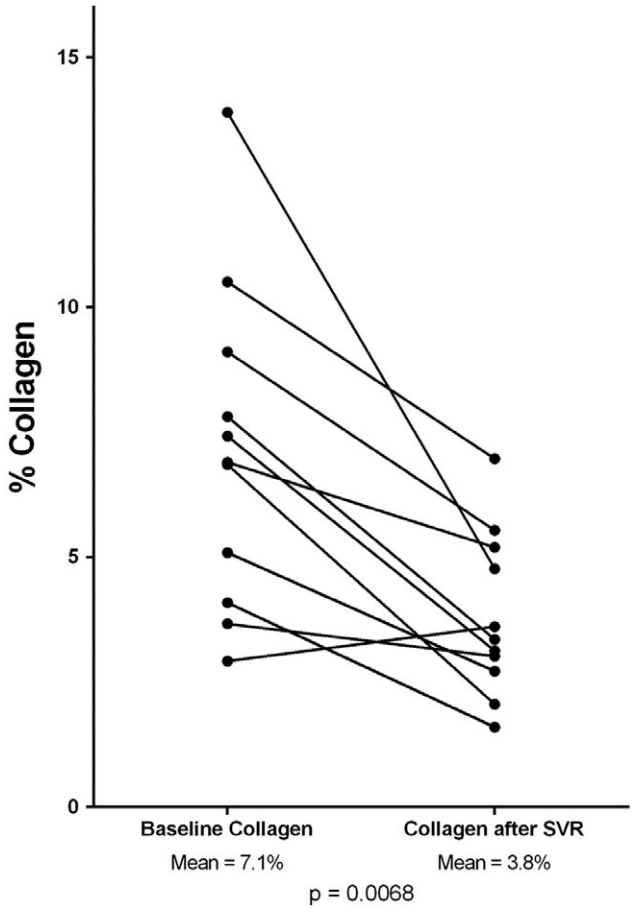

FIG. 4. Collagen quantitation morphometric analysis before and after SVR.

DAA therapy is overestimated by TE compared to liver biopsy, which remains the only reliable and practical approach to stage liver fibrosis after SVR is achieved.

HCC screening should still be considered in patients with HCV status without cirrhosis post-SVR after DAA therapy despite their liver stiffness being <9.5 kPa because they may continue to have F3 fibrosis and thus be at increased risk of HCC development. In this study, 4 patients were found to have HCC. The 2 patients who had cirrhosis at baseline did not demonstrate liver stiffness improvement, but the 2 patients who were F3 with HCC diagnosed at 6 months and 2 years after therapy demonstrated liver stiffness improvement consistent with F0-F1 staging.

The sensitivity and specificity of TE to determine the correct fibrosis stage typically ranges from 78% to 89% depending on the actual stage seen on liver biopsy in patients with viremia and HCV.[17] Although there is a range of cutoffs that may be used for fibrosis stages, the discordance between TE and liver biopsy after SVR from DAA therapy in this study exceeds expectations (Fig. 3). These findings suggest that a distinction must be made between total amount of hepatic fibrosis reflected in liver stiffness measurement and the histopathologic features seen on liver biopsies used in staging.

In the present study, the repeat biopsies of 7 patients revealed major reductions in sinusoidal fibrosis (Supporting Figures S2 and S3). Sinusoidal fibrosis regression was recently correlated with patients who had significant TE decreases in a study where liver transplant recipients underwent DAA therapy for

recurrent HCV.[22] Current histologic staging systems do not use sinusoidal fibrosis, which has been traditionally associated with nonalcoholic steatohepatitis and perivenular fibrosis. However, HCV is associated with sinusoidal fibrosis, which has been suggested to be an early histopathologic sign of HCV recurrence in liver transplant recipients.[23] The reduction in sinusoidal fibrosis may reflect an overall reduction in total collagen and hepatic fibrosis; sinusoidal fibrosis has previously been shown to be an independent variable that correlates with hepatic fibrosis.[24] This hypothesis is further supported by the morphometric analysis that was completed on the first 11 biopsied patients from the present study, where all but 1 had significant reductions in total collagen. Although there may be sampling variability, morphometric analysis has been shown to be a more sensitive tool for tracking changes in fibrosis than numerical scoring systems.[25] Morphometry is a more specific and quantifiable measurement, but the lack of its wide-spread availability makes it an impractical test for clinical use.

The limitations of this study include limited sample size of patients undergoing a repeat biopsy and consequently possible sampling error. It is feasible that a larger study would show a closer correlation between TE scores <9.5 kPa after SVR and resolution of advanced fibrosis or cirrhosis. However, it may prove impractical or even impossible to convince a significant number of patients who have accomplished a virologic cure and have no evidence of portal hypertension to undergo a liver biopsy years after SVR. Necro-inflammation and transaminase flares before treatment could lead to an increase of stiffness not related to liver fibrosis (confounders), and these data were not captured in this trial. However, acute inflammation is unlikely to have a role in the TE and biopsy changes because the findings were consistent over a prolonged period during normal AST/ALT levels (Fig. 2) and because the median time to follow-up biopsy was 3 years. Unfortunately, no TE studies were performed immediately after DAA therapy, but previous studies have shown that there is an immediate improvement of 2 kPa or more due to resolution of inflammation during antiviral treatment.[22,26] TE studies were not conducted prior to antiviral therapy in patients who were treated before 2013 when FibroScan was approved for use in the United States. This limited the ability to assess TE measurement and histology before therapy, but the aim of this study was to assess fibrosis reversal after therapy. Multiple studies have previously shown that this correlation in chronic HCV before therapy is valid[1-3,14,15,17,18]; 109 patients who were treated with sofosbuvir-based regimens and enrolled after September 2015 are still being followed. Although this is a limitation to the current report, the liver biopsies are being delayed for 3 years to maximize the opportunity of seeing a clinically meaningful reversal in fibrosis in the patients who agree to have the procedure done.

In this study, significant improvements in liver stiffness consistent with at least one stage of fibrosis occurred in 62% of patients with HCV with advanced fibrosis or cirrhosis after SVR achieved by DAA therapies, with a mean follow-up of 2 years. This is the first study that has also paired liver biopsies showing a regression of liver fibrosis with morphometry after DAA. By obtaining follow-up liver biopsies on a select, albeit small, group of patients with dramatic TE improvements, this study demonstrated that these TE-predicted improvements, especially with cutoffs of <9.5 kPa, were not seen in histologic staging but in morphometric analysis. Of the 13 patients who had a repeat biopsy and displayed significant improvement in liver stiffness (<9.5 kPa), only 31% showed improvement to less than F3 staging on pathology. There is discordance between the level of liver stiffness improvement measured by TE and fibrosis regression seen on liver biopsies using these simple histologic scoring systems. Morphometry demonstrates 46% reduction in fibrosis with SVR over a relatively short time period and is a more accurate measure of improvement in fibrosis regression. The significance of sinusoidal fibrosis regression will need to be further investigated.

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Author names in bold designate shared co-first authorship.