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ORIGINAL ARTICLE

Non-invasive assessment of fibrosis regression and portal hypertension in patients with advanced chronic hepatitis C virus (HCV)-associated liver disease and sustained virologic response (SVR): 3 years follow-up of a prospective longitudinal study

Viola Knop[1] | Daniel Hoppe[1,2] | Johannes Vermehren[1] | Sven Troetschler[1,3] | Eva Herrmann[4] | Annika Vermehren[1] | Mireen Friedrich-Rust[1] | Christoph Sarrazin[1,5] | Jonel Trebicka[1] | Stefan Zeuzem[1] | Martin-Walter Welker[1]

[1]Medizinische Klinik 1, Universitätsklinikum Frankfurt, Frankfurt am Main, Germany

[2]St. Elisabeth-Krankenhaus, Leipzig, Germany

[3]Ketteler Krankenhaus, Offenbach, Germany

[4]Institut für Biostatistik und mathematische Modellierung, Goethe-Universität Frankfurt, Frankfurt am Main, Germany

[5]St-Josefs-Hospital, Wiesbaden, Germany

Correspondence
Martin-Walter Welker, Universitätsklinikum Frankfurt, Medizinische Klinik 1, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany.
Email: Welker@med.uni-frankfurt.de

Abstract

Long-term effects on cirrhosis and portal hypertension of direct antiviral agent (DAA)-based eradication of hepatitis C virus (HCV) are still under debate. We analysed dynamics of liver and spleen elastography to assess potential regression of cirrhosis and portal hypertension 3 years post-treatment. Fifty-four patients with HCV-associated cirrhosis and DAA-induced SVR were included. Liver and spleen stiffness were measured at baseline (BL), end of treatment (EOT), 24 weeks after EOT (FU24) and 1, 2 and 3 (FU144) years post-treatment by transient liver elastography (L-TE) and point shear wave elastography (pSWE) using acoustic radiation force impulse (ARFI) of the liver (L-ARFI) and spleen (S-ARFI). Biochemical, virological and clinical data were also obtained. Liver stiffness assessed by L-TE decreased between BL [median (range), 32.5(9.1–75) kPa] and EOT [21.3(6.7–73.5) kPa; $p < .0001$] and EOT and FU144 [16(4.1–75) kPa; $p = .006$]. L-ARFI values improved between EOT [2.5(1.2–4.1) m/s] and FU144 [1.7(0.9–4.1) m/s; $p = .001$], while spleen stiffness remained unchanged. Overall, L-TE improved in 38 of 54 (70.4%) patients at EOT and 29 of 38 (76.3%) declined further until FU144, whereas L-ARFI values decreased in 30/54 (55.6%) patients at EOT and continued to decrease in 28/30 (93.3%) patients at FU144. Low bilirubin and high albumin levels at BL were associated with improved L-ARFI values ($p = .048$) at EOT or regression of cirrhosis (<12.5 kPa) by L-TE at FU144 ($p = .005$), respectively. Liver stiffness, but not spleen stiffness, continued to decline in a considerable proportion of patients with advanced liver disease after HCV eradication.

Abbreviations: ALT, alanine aminotransferase; APRI, aspartate aminotransferase/platelet ratio index; ARFI, acoustic radiation force impulse; AST, aspartate aminotransferase; BL, baseline; BMI, body mass index;; CPT, Child-Pugh-Turcotte; DAA, direct-acting antiviral agent (s); EOT, end of treatment; FU144, follow-up 144 weeks after end of treatment; FU24, follow-up 24 weeks after end of treatment; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HVPG, hepatic venous pressure gradient; INR, international normalized ratio; kPa, kilopascal; L-ARFI, liver ARFI; LSM, liver stiffness measurement; L-TE, transient elastography of the liver;; MELD, model of end-stage liver disease; PH, portal hypertension; pSWE, point shear wave elastography; S-ARFI, spleen ARFI; SCD, skin capsule distance; SVR, sustained virologic response.

KEYWORDS
acoustic radiation force impulse, chronic hepatitis C, direct-acting antiviral (DAA) treatment, liver cirrhosis, portal hypertension, transient elastography

1 | INTRODUCTION

Antiviral therapy of chronic hepatitis C with direct-acting antiviral agents [DAA(s)] results in >90% sustained virologic response (SVR) rates including patients with compensated liver cirrhosis.[1,2] The development of DAA-based, interferon-alfa free antiviral therapy in patients with hepatitis C enabled treatment even in patients with (decompensated) cirrhosis or prior liver transplantation.[3–5] However, there is still lack of knowledge to what extent cirrhosis including portal hypertension is reversible after HCV eradication in patients with HCV- associated cirrhosis.[6–10]

In a previous report on our study cohort, we demonstrated an improvement of liver stiffness values between baseline (BL) and end of treatment (EOT) as well as between BL and 24 weeks after EOT (FU 24) in patients with HCV-associated advanced liver disease and DAA-induced SVR.[11] Although liver biopsy has been considered the gold standard for the assessment of fibrosis status, it is limited by its rare but potential severe complications. In addition, the accuracy of assessment of cirrhosis by liver biopsy is decreased due to sampling variations, inadequate specimen size and observer variability.[12] In recent years, non-invasive methods have been evaluated for the indirect assessment of liver fibrosis and portal hypertension.[13,14] Technologies such as transient elastography or point shear wave elastography (pSWE) using acoustic radiation force impulse (ARFI) have revolutionized the monitoring of patients in daily clinical practice. Transient elastography of the liver (L-TE) and ARFI of the liver (L-ARFI) measure liver stiffness, which correlates with fibrosis.[15–17] Regression of liver fibrosis was shown in patients treated with interferon up to 4 years after treatment, particularly in those who achieved SVR.[18,19] In recent years, studies analysing the effect of interferon-free DAA regimens on fibrosis regression have demonstrated short-term reduction in liver stiffness by different elastography techniques within 18 months post-treatment.[20–23] However, studies with follow-up intervals beyond several years are scarce.

A number of studies have investigated spleen stiffness as a non-invasive parameter for portal hypertension.[24–26] In a recent trial, elevated spleen stiffness measured by ARFI (S-ARFI) represented a reliable non-invasive tool for the prediction of oesophageal varices in HCV-infected patients.[27]

The aim of the present study was to assess the frequency and degree of fibrosis regression as well as reduction of portal hypertension by liver and spleen elastography in patients with HCV-associated advanced liver disease and DAA-induced SVR up to 3 years post-treatment.

2 | MATERIALS AND METHODS

2.1 | Patient selection and study design

Treatment of chronic hepatitis C within the present prospective study was conducted between 06/2014 and 12/2015 at a German tertiary care centre. The study was performed in accordance with the Declaration of Helsinki and the ICH/CPMP guidelines 'Good Clinical Practice' and was approved by the local ethics committee. Written informed consent was obtained from all participants before enrolment. Here, we report the 3 years follow-up update to the initial study report.[8] Main inclusion criteria of the initial study and thus the current analysis were confirmation of HCV infection by anti-HCV (third-generation) enzyme immunoassay (Roche Diagnostics, Mannheim, Germany), quantifiable HCV-RNA by

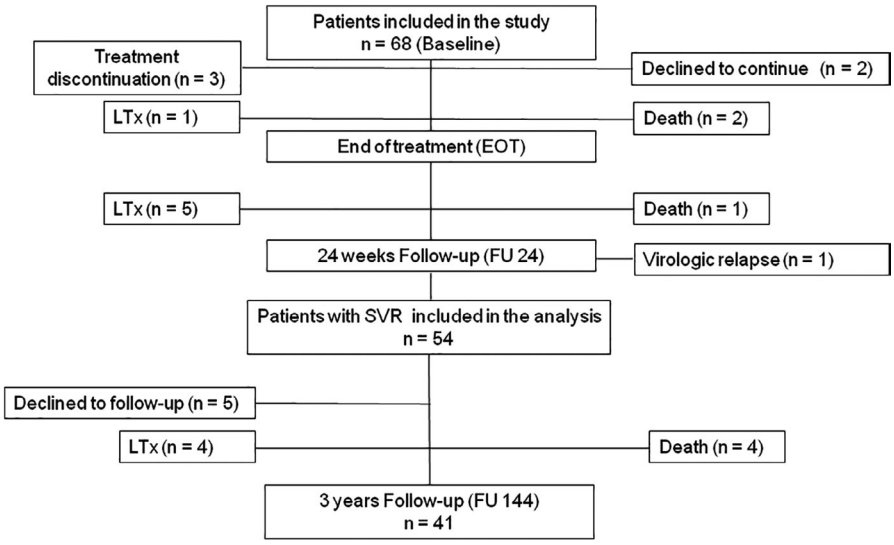

FIGURE 1 The flow diagram illustrates selection of patients analysed in the study

quantitative reverse transcription polymerase chain reaction assay [COBAS TaqMan HCV test 2.0 (Roche Diagnostics, Mannheim, Germany)], presence of cirrhosis determined by histology, if available, abdominal ultrasound and/or transient elastography (stiffness values ≥12.5 kPa), and timely planned DAA-based, interferon-free antiviral therapy. Patients with concomitant liver diseases, including autoimmune disorders, coinfection with hepatitis B virus, Wilson's disease, hemochromatosis, systemic infections, preexisting severe psychiatric conditions, significant alcohol consumption (≥40 g/day in women and ≥60 g/day in men) and drug abuse within the past year were excluded from the study. Details of the study design have been reported previously as well as results regarding fibrosis regression and portal hypertension of these patients at end of treatment (EOT) and again at 24 weeks after EOT (FU24).[8] A synopsis of the study design including the initial and the current follow-up report is given in Figure 1.

Clinical and laboratory assessments including routine biochemical liver tests were obtained at baseline (BL) of antiviral therapy, EOT, FU24 as well as at 1, 2 and 3 years post-treatment. Moreover, MELD score[28] and CPT score[29] were calculated for each patient. APRI Score [AST level (ULN)/(platelet counts (10^9/l) × 100)][30] was available in a subpopulation (n = 33) at BL, EOT and 3 years post-treatment and was performed retrospectively. Measurements of liver and spleen stiffness were performed at BL, EOT and FU24 as well as at 1, 2 and 3 years post-treatment. In addition, spleen diameter and the presence or absence of ascites were evaluated by conventional B-mode ultrasound.

The choice of antiviral therapy was at the physician's discretion. In detail, all 54 patients included in the analysis received a DAA-based, interferon-free therapy regimen with or without ribavirin for 12 or 24 weeks (Table 1). Treatment details of 43/54 patients were recorded within the European compassionate use programme (Clinical Trials.gov Identifier NCT02097966). Data of those patients have in part been or will be published within reports of the respective programme.[31] Moreover, clinical data of three patients were reported within a study focusing on complications before and during antiviral therapy.[32]

TABLE 1 Patients' characteristics, virological data and antiviral treatment

Parameter *n* or median (range)	Patients (*n* = 54)
Age (years)	57 (30–86)
Gender male/female (*n*/*n*)	37/17
Ethnicity (*n*)	
Caucasian	48
Non caucasian	6
HIV coinfection (*n*)	2
Prior or current HCC (*n*)[a]	2
Prior liver transplantation (*n*)	5
Ascites (*n*)	4
Body mass index (kg/m^2)	26.1 (19–44)
Bilirubin (mg/dl)	1.1 (0.4–5.4)
Albumin (g/dl)	3.7 (2.8–4.9)
INR	1.13 (0.9–1.5)
AST (U/l)	82 (17–260)
ALT (U/l)	71 (17–254)
Platelets (/nl)	90 (28–223)
APRI Score (AST * 100/109/l)	2.5 (0.3–15)
CTP Score	5 (5–9)
MELD Score	9 (6–17)
HCV genotype distribution 1a/1b/3/4 (*n*)	19/17/13/5
Baseline HCV RNA ($\log_{10}$ IU/ml)	6.02 (4.19–7.01)
Liver FibroScan (kPa)[b]	32.4 (9.1–75)
Liver ARFI (m/s)	2.7 (1.2–4.1)
Spleen ARFI (m/s)	3.4 (2–4.3)
Spleen diameter (cm)	14.7 (9–21)
Treatment details	
Daclatasvir +Sofosbuvir ± Ribavirin (*n*)	36
Sofosbuvir +Ledipasvir ± Ribavirin (*n*)	12
Simeprevir +Sofosbuvir ± Ribavirin (*n*)	5
Ombitasvir/Paritaprevir/ Ritonavir + Dasabuvir (*n*)	1

Abbreviations: ALT, alanine aminotransferase; ARFI, acoustic radiation force impulse; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma.

[a]Patients had prior radiofrequency ablation.

[b]In 4 of 54 patients, liver FibroScan was not applicable due to ascites.

2.2 | Transient elastography (TE)

Patients were placed on a supine position with elevated arms above their heads. Liver transient elastography (L-TE) was carried out with the FibroScan® 502 Touch device (Echosens, Paris, France) which incorporates an ultrasound transducer probe mounted on the axis of a vibrator. The vibrator generates painless vibration with a frequency of 50 Hz, which leads to an elastic shear wave propagating through the skin and the subcutaneous tissue to the liver. Shear wave velocity (expressed in kilopascal, kPa) is directly related to the stiffness of the tissue.[33] Stiffness measurements were performed with a 3.5-MHz transducer ('M-probe') in patients with a skin capsule distance (SCD) ≤25 mm and with a 2.5-MHz transducer ('XL-probe') in patients with a SCD >25 mm, respectively. The fasting interval between last food intake and measurement was assigned to at least 4 h.[34] Ten measurements were taken to obtain the median number in kilopascal. Unreliable measurements were defined as an interquartile range (IQR) to median value ratio greater than 30% and a success rate (SR) less than 60%. These thresholds were chosen, because they were reported to improve inter-variability and intra-variability discrepancies.[35,36] For diagnosing liver cirrhosis, the cut-off value of 12.5 kPa was used.[37]

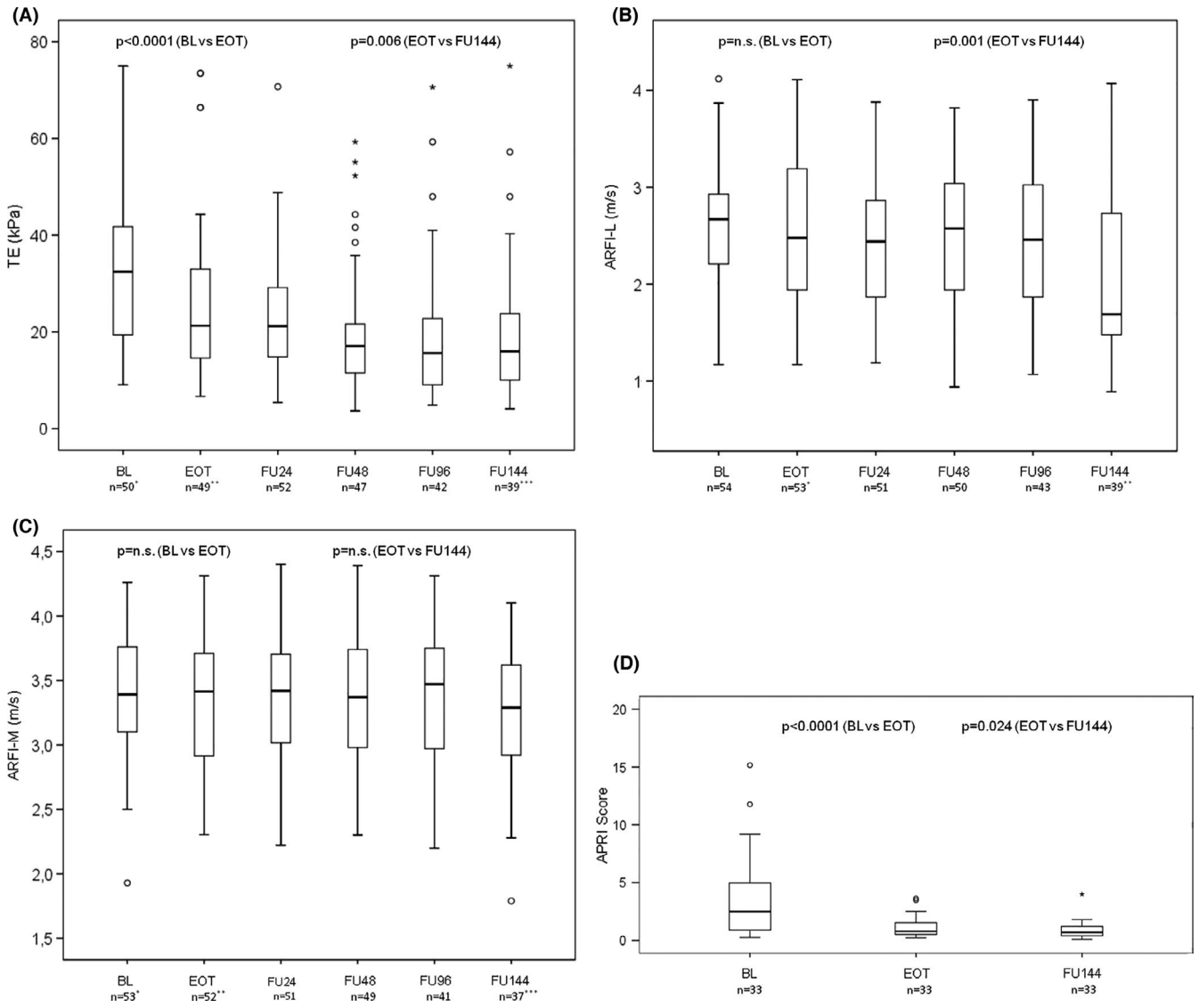

FIGURE 2 (A). Changes of liver stiffness assessed by L-TE between baseline (BL) and 3 years post-treatment (FU144). At BL (*), L-TE was not applicable in 4 patients due to ascites. At EOT (**), L-TE was not feasible in 1 patient due to ascites. At FU144 (***), L-TE was not applicable in 2 patients due to increased BMI. 13 patients had premature termination of the study. Whisker and box plots represent medians, first and third quartiles and range. (B) Changes of liver stiffness assessed by L-ARFI between baseline (BL) and 3 years post-treatment (FU144). At EOT (*), 1 patient refused L-ARFI. At FU144 (**), L-ARFI was not applicable in 1 patient due to increased BMI. 1 patient refused L-ARFI. 13 patients had premature termination of the study. Whisker and box plots represent medians, first and third quartiles and range. (C) Changes of spleen stiffness assessed by S-ARFI between baseline (BL) and 3 years post-treatment (FU 144). At BL (*), S-ARFI was not applicable in 1 patient due to splenectomy. At EOT (**), S-ARFI was not feasible in 1 patient due to splenectomy. 1 patient refused S-ARFI. At FU144 (***), S-ARFI was not applicable in 1 patient due to increased BMI. 2 patients had prior splenectomy. 1 patient refused S-ARFI. 13 patients had premature termination of the study. Whisker and box plots represent medians, first and third quartiles and range. (D) Changes of APRI Score between baseline (BL) and 3 years post-treatment (FU 144) in a subgroup of patients ($n = 33$). 13 patients had premature termination of the study. In 8 patients, we had missing values. Whisker and box plots represent medians, first and third quartiles and range

2.2.1 | Point shear wave elastography (pSWE) using acoustic radiation force impulse (ARFI)

Patients were placed on a supine position with elevated arms above their heads. Liver (L-ARFI) and spleen (S-ARFI) ARFI were performed with a Siemens AcusonS2000™ (Siemens AG, Erlangen, Germany) ultrasound system. The ultrasound probe produces an acoustic 'push' impulse that generates shear waves, which propagate into the tissue. Their speed is measured in metres per second (m/s). The propagation speed increases with fibrosis severity.[16,38,39] Ten measurements were carried out for liver and spleen stiffness, respectively, on the right liver lobe as well as at the level of the lower pole/middle third of the spleen. The measurement box was placed 2 cm deep in projection on the hepatic and splenic parenchyma, respectively. The recommended examination standards published by Karlas et al.[40] were used to minimize the overestimation of spleen

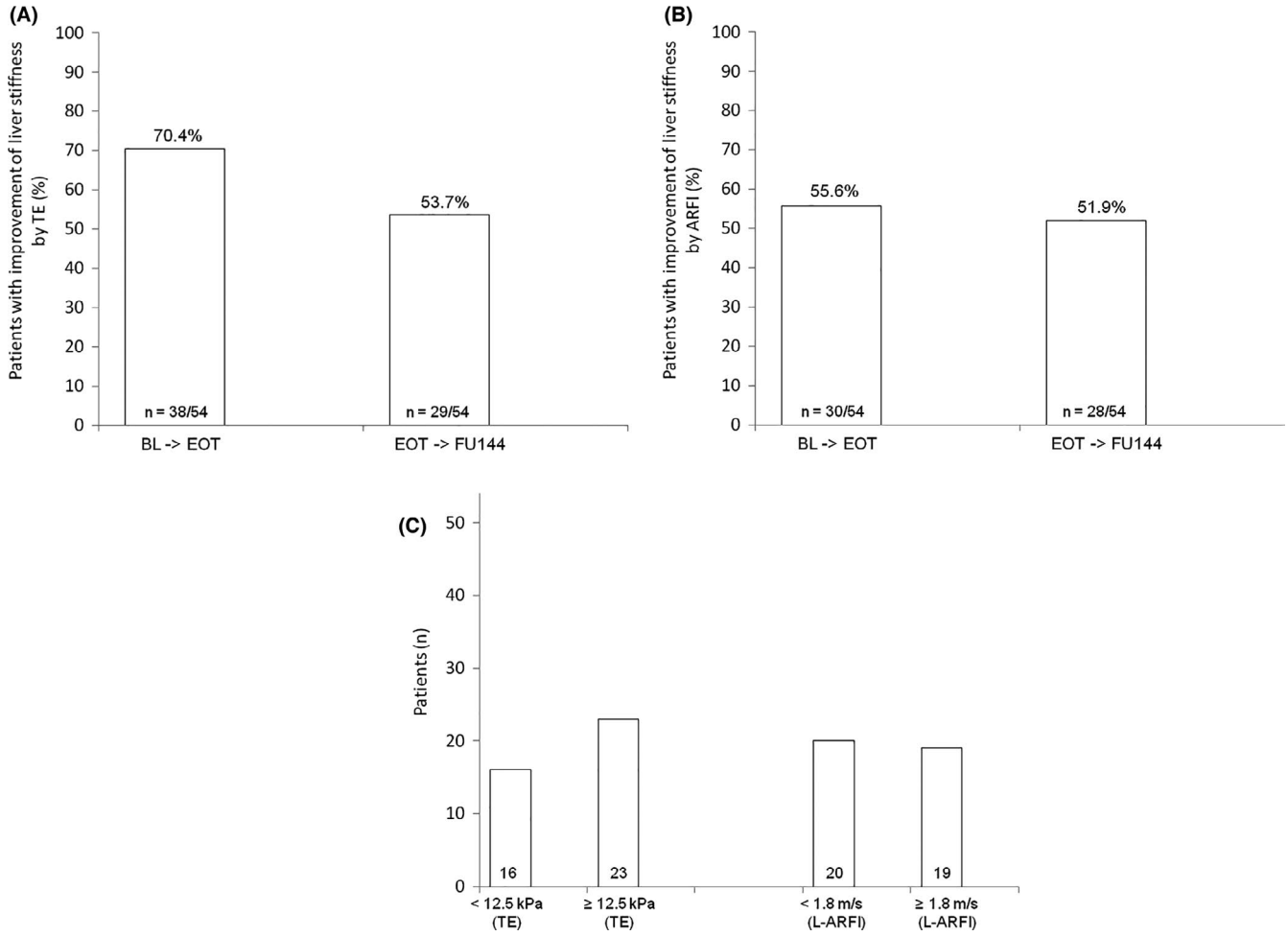

FIGURE 3 (A) Improvement of liver stiffness assessed by TE between BL and EOT as well as between EOT and FU144. Changes are given in absolute LSM value. (B) Improvement of liver stiffness assessed by ARFI between BL and EOT as well as between EOT and FU144. Changes are given in absolute LSM value. (C) Patients were classified to be non-cirrhotic (<12.5 kPa; <1.8 m/s) or cirrhotic (≥12.5 kPa; ≥1.8 m/s) by TE and ARFI methods at FU144

stiffness values. An L-ARFI cut-off value of 1.8 m/s (or higher) was used to determine presence or absence of cirrhosis.[38]

2.2.2 | Ascites and splenic length diameter measurement

Ascites and splenic length measurement, presence of ascites and splenic length diameter were obtained by use of a Siemens AcusonS2000™ (Siemens AG) ultrasound system.

2.2.3 | Statistical analyses

Data were expressed as median and range for continuous variables in addition to both frequency and percentage for categorical variables. Box plots of liver and spleen stiffness values as well as APRI Score were provided at different time points between BL and three years post-treatment illustrating median changes of individual patient data shown as absolute values. Wilcoxon test was applied for comparison of two paired groups. Univariate and multivariate regression analyses were performed to identify baseline factors (continuous variables) as predictors of liver stiffness improvement. All tests were two-sided, and statistical significance was defined as $p < .05$. Data were analysed by using the statistical software package SPSS 24 for Windows.

3 | RESULTS

3.1 | Patient characteristics

Between June 2014 and December 2015, a total of 54 patients who had achieved SVR to DAA-based therapy were included in the present analysis. Three-year follow-up data (FU144) were available in 41 patients (refusal of follow-up, $n = 5$; liver transplantation, $n = 4$; fatal outcome, $n = 4$ (Figure 1)). Main baseline characteristics are given in Table 1. A minority of patients (2 of 54) was co-infected with human immunodeficiency virus (HIV), and 2 of 54 had a history of HCC prior to antiviral therapy.

TABLE 2 Univariate and multivariate analysis of baseline factors for association with improvement of liver stiffness by TE between BL and EOT as well as between EOT and FU144

	BL->EOT				EOT->FU144			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*
Gender	0.985 (0.280–3.469)	>.05		>.05	0.956 (0.302–3.023)	>.05		>.05
Age	1.007 (0.952–1.066)	>.05		>.05	0.967 (0.916–1.021)	>.05		>.05
BMI	0.976 (0.874–1.089)	>.05		>.05	1.024 (0.923–1.135)	>.05		>.05
Bilirubin	0.615 (0.330–1.147)	>.05		>.05	1.137 (0.640–2.018)	>.05		>.05
Albumin (*high*)	1.466 (0.499–4.304)	>.05		>.05	0.300 (0.102–0.883)	.029		>.05
AST	1.009 (0.997–1.022)	>.05		>.05	0.997 (0.987–1.006)	>.05		>.05
ALT	1.007 (0.996–1.019)	>.05		>.05	0.995 (0.986–1.005)	>.05		>.05
INR	0.367 (0.008–17.558)	>.05		>.05	22.964 (0.503–1048.047)	>.05		>.05
Platelets	0.995 (0.984–1.006)	>.05		>.05	0.996 (0.985–1.007)	>.05		>.05
Genotype 1	0.769 (0.227–2.610)	>.05		>.05	0.894 (0.287–2.787)	>.05		>.05

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BL, baseline; EOT, end of treatment; FU144, 144 weeks post-treatment; INR, international normalized ratio; TE, transient elastography.

3.1.1 | *Changes of liver and spleen stiffness as well as APRI Score between baseline (BL) and 3 years post-treatment (FU 144)*

As shown in Figure 2A, asignificant decline of liver stiffness assessed by L-TE was observed between BL [median (range), 32.5 (9.1–75) kPa] and EOT [median (range), 21.3 (6.7–73.5) kPa; $p < .0001$], and there was continued improvement between EOT and FU144 [median (range), 16 (4.1–75) kPa; $p = .006$].

A significant reduction in liver stiffness measured by L-ARFI was observed between EOT [median (range), 2.5 (1.2–4.11) m/s] and FU144 [median (range), 1.7 (0.9–4.1) m/s; $p = .001$] (Figure 2B). However, no significant decrease of L-ARFI values was found between BL [median (range), 2.7 (1.2–4.1) m/s] and EOT [median (range), 2.5 (1.2–4.1) m/s; $p = .278$].

Spleen stiffness assessed by S-ARFI did not show any significant changes between BL [median (range), 3.4 (1.9–4.3) m/s] and EOT [median (range), 3.4 (2.3–4.3) m/s; $p = .74$] or FU144 [median (range), 3.3 (1.8–4.1) m/s; $p = .138$)] (Figure 2C).

A subanalysis of $n = 33$ patients revealed a significant decrease in APRI score between BL [median (range), 2.5 (0.3–15)] and EOT [median (range), 0.8 (0.2–3.6); $p < .0001$] with continued improvement between EOT and FU144 [median (range), 0.7 (0.1–4); $p = .024$] (Figure 2D).

Changes of biochemical markers, such as ALT, platelets and albumin, are shown in Supplement Figure 1.

3.1.2 | Improvement of liver stiffness by TE and ARFI methods between BL and FU 144

Overall, 38 of 54 patients (70.4%) achieved improvement of liver stiffness by TE at EOT and 29 of 38 (76.3%) declined further until FU144 (Figure 3A).

By L-ARFI, 30 of 54 patients (55.6%) presented a decrease in liver stiffness at EOT and 28 of 30 (93.3%) continued to improve until FU144 (Figure 3B).

At FU144, 16 and 20 patients were classified to be non-cirrhotic by TE (<12.5 kPa) and ARFI (<1.8 m/s) methods compared to 23 and 19 patients with estimated cirrhosis (≥12.5 kPa; ≥1.8 m/s) (Figure 3C).

3.1.3 | Univariate and multivariate analysis of baseline factors for association with improvement of liver stiffness by TE and ARFI methods between BL and FU 144

Univariate analysis indicated that high albumin was the only baseline parameter that predicts reduction in liver stiffness by TE between EOT and FU144 ($p = .029$) (Table 2).

Moreover, low total bilirubin serum levels ($p = .048$) and low INR values ($p = .048$) at BL were associated with improvement of stiffness between BL and EOT measured by L-ARFI. By multivariate

TABLE 3 Univariate and multivariate analysis of baseline factors for association with improvement of liver stiffness by ARFI between BL and EOT as well as between EOT and FU144

	BL-> EOT				EOT->FU144			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*
Gender	1.244 (0.390–3.970)	>.05		>.05	0.938 (0.297–2.963)	>.05		>.05
Age	1.027 (0.973–1.084)	>.05		>.05	1.024 (0.972–1.080)	>.05		>.05
BMI	0.987 (0.890–1.095)	>.05		>.05	0.998 (0.900–1.106)	>.05		>.05
Bilirubin (*low*)	0.452 (0.206–0.994)	.048	0.452 (0.206–0.994)	.048	0.920 (0.519–1.630)	>.05		>.05
Albumin	2.088 (0.742–5.875)	>.05		>.05	2.047 (0.748–5.603)	>.05		>.05
AST	1.003 (0.993–1.014)	>.05		>.05	1.002 (0.992–1.011)	>.05		>.05
ALT	1.006 (0.996–1.016)	>.05		>.05	1.004 (0.995–1.013)	>.05		>.05
INR (*low*)	0.019 (0.000–0.961)	.048		>.05	0.750 (0.002–3.226)	>.05		>.05
Platelets	1.005 (0.994–1.016)	>.05		>.05	1.003 (0.993–1.014)	>.05		>.05
Genotype 1	0.667 (0.212–2.095)	>.05		>.05	1.250 (0.401–3.894)	>.05		>.05

Abbreviations: 144 weeks post-treatment; ALT, alanine aminotransferase; ARFI, acoustic radiation force impulse; AST, aspartate aminotransferase; BL, baseline; EOT, end of treatment, FU144; INR, international normalized ratio.

analysis, only bilirubin remained significantly associated with improved L-ARFI values ($p = .048$) (Table 3).

As shown in Table 4, high albumin (p=0.004) and high platelets at BL (p=0.029) predicted non-cirrhotic status (<12.5 kPa) by TE at FU144. By multivariate analysis, only albumin remained significantly associated with non-cirrhotic status (<12.5 kPa) 3 years post-treatment ($p = .005$).

4 | DISCUSSION

The development of DAA-based, interferon-free antiviral treatment regimens characterized by excellent efficacy and tolerability has rendered possible eradication of HCV even in patients with advanced liver disease including decompensated cirrhosis. Yet, the long-term clinical benefit after successful therapy in the latter has not been fully evaluated, and the issue of regression as well as reversibility of cirrhosis and portal hypertension remains controversial.[7–10,41–46]

In the current study, we investigated dynamics of liver and spleen stiffness assessed by two different elastography tools over three years post-treatment in patients with liver cirrhosis and DAA-induced SVR. Thus, 19% of the patients included in the trial had Child-Pugh-Turcotte (CPT) score B or C cirrhosis. To the best of our knowledge, the present study is the first analysis of longitudinal liver and spleen stiffness measurements over three years after EOT in this context.

As reported previously,[11] we observed a two-phase decrease in liver stiffness after DAA- based antiviral therapy in patients with advanced liver disease consisting of a rapid decline already during treatment and a continued improvement post-treatment in more than 50% of our patients. The early decline—pronounced between BL and EOT—might be influenced rather by reduction in necro-inflammatory activity than by fibrosis regression. In this current follow-up study, we found a significant decrease in liver stiffness between EOT and FU144. Even in the post-treatment phase, we could demonstrate continual improvement in liver stiffness, most likely reflecting ongoing fibrosis reduction. However, it has to be pointed out that fibrosis regression seems to proceed much more slowly after antiviral therapy has been terminated. Similarly, we observed continual reduction in APRI score and albumin levels post-treatment strengthening the causal relationship with fibrosis regression. It has to be stressed that elastography dynamics indicated reversal of cirrhosis in about one third of patients three years after end of antiviral therapy compared to BL. Although the suitable definition of cirrhosis regression by liver elastography is still under debate in nonviremic HCV-cured patients, our data are in line with other reports investigating fibrosis regression by histological assessment. Improvement of liver stiffness post-treatment may be overrated by elastography in comparison with histological staging.[16,42] However, both above-mentioned histology-based studies also demonstrated a reduction of fibrosis stage in a proportion of patients with SVR.[16,42] By morphometric analysis, Pan et al.[42] found that a substantial number of patients with reversal of advanced fibrosis or cirrhosis assessed by

TABLE 4 Univariate and multivariate analysis of baseline factors for association with non-cirrhotic status by TE (<12.5 kPa) and ARFI (<1.8 m/s) at FU144

	<12.5 kPa at FU144				<1.8 m/s at FU144			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*	Odds ratio (95% CI)	*p*
Gender	0.625 (0.151–2.586)	>.05		>.05	0.735 (0.193–2.793)	>.05		>.05
Age	0.957 (0.898–1.019)	>.05		>.05	1.013 (0.957–1.073)	>.05		>.05
BMI	1.072 (0.939–1.244)	>.05		>.05	1.104 (0.966–1.262)	>.05		>.05
Bilirubin	1.361 (0.632–2.932)	>.05		>.05	0.798 (0.415–1.537)	>.05		>.05
Albumin (high)	0.107 (0.023–0.494)	.004	0.107 (0.023–0.502)	.005	0.408 (0.127–1.314)	>.05		>.05
AST	1.003 (0.992–1.014)	>.05		>.05	0.993 (0.982–1.005)	>.05		>.05
ALT	0.999 (0.989–1.009)	>.05		>.05	0.993 (0.983–1.004)	>.05		>.05
INR	96.869 (0.428–21928.72)	>.05		>.05	3.862 (0.40–373.879)	>.05		>.05
Platelets (high)	0.984 (0.970–0.998)	.029		>.05	0.995 (0.982–1.008)	>.05		>.05
Genotype 1	0.271 (0.063–1.168)	>.05		>.05	0.495 (0.118–2.081)	>.05		>.05

Abbreviations: ALT, alanine aminotransferase; ARFI, acoustic radiation force impulse; AST, aspartate aminotransferase; BL, baseline; EOT, end of treatment; FU144, 144 weeks post-treatment; INR, international normalized ratio; TE, transient elastography.

TE had a marked reduction in collagen content and a regression in sinusoidal fibrosis. Moreover, it has been previously reported that HCV is associated with sinusoidal fibrosis in terms of early histopathologic sign of HCV recurrence in liver transplant recipients.[47] A study by Chevalier et al.[48] demonstrated that sinusoidal fibrosis was an independent variable that correlates well with hepatic fibrosis. Therefore, the reduction of liver stiffness shown in our study indicating reversal of cirrhosis in given patients seems comprehensible in the context of other trials.

In contrast to the continued decline of liver stiffness, we did not observe significant changes of spleen stiffness, which is considered a non-invasive marker for clinical portal hypertension. Thus, the data of our study suggest that portal hypertension may persist in patients with advanced liver disease despite eradication of HCV and despite subsequent regression of liver fibrosis. This is in accordance with current considerations indicating that portal hypertension is not exclusively a result of mechanical effects of fibrosis but depends on a complex interplay of vasodilatation and vasoconstriction factors.[49] So far, there are only a few trials investigating changes in spleen stiffness in patients who achieved SVR.[50–52] Moreover, post-treatment follow-up was relatively short (12 to 48 weeks) in these studies. Verlinden et al.[50] observed no effect on spleen stiffness by real-time shear wave elastography in patients with advanced fibrosis, whereas Pons et al.[51] documented a rapid decline within 4 weeks by TE with no further changes until SVR 48 in patients with compensated liver disease. Interestingly, Ravaioli et al.[52] could show that reduction of spleen stiffness was more evident in patients without clinically significant portal hypertension. Thus, clinically significant portal hypertension occurring in association with extrahepatic haemodynamic factors[53] is more likely to persist for a longer period after eradication of HCV. Similar results were obtained by a multicentre prospective study by Lens et al indicating that hepatic venous pressure gradient (HVPG) remained significantly elevated in 78% of patients at SVR 24.[54] This is in accordance with Mandorfer et al.[8] detecting that HVPG decrease was less likely in patients with more advanced liver dysfunction.

Our observation of persistent increased spleen stiffness despite HCV eradication and even regression of liver stiffness and thus cirrhosis is of clinical importance. A previous report demonstrated that risk of HCC development remained clinically meaningful in patients with portal hypertension complicated with advanced fibrosis even after eradication of HCV.[55] Patients with clinical significant portal hypertension, assessed by HVPG measurement, have been shown to remain at risk for liver decompensation within the first 5 years after antiviral treatment.[45] Thus, our data confirm the need for continuing HCC surveillance and stress the necessity for surveillance of complications of portal hypertension in HCV-cured patients with advanced liver disease despite improvement of liver function and liver stiffness.

Finally, we analysed putative associations of clinical and biochemical parameters and regression of liver stiffness. Interestingly, neither ALT nor AST baseline levels were shown to be associated with liver stiffness improvement, suggesting that reduction in elastography values might not exclusively be reflected by diminishing necroinflammation.

Contrary to other studies, liver stiffness reduction was not influenced by baseline BMI in our patients. Soliman et al.[56] found that fibrosis scores decreased more pronounced in patients with lower BMI at baseline. However, in this study, patients had higher BMI values overall, and the proportion of liver disease-associated cirrhosis was only 25%. Moreover, we could show that known surrogate parameters for advanced cirrhosis, such as high bilirubin, high INR, low albumin and low platelets, were predictive factors for the lack of fibrosis regression.

The results of our current study are in accordance with other studies in the field.[20–23] Moreover, we provide new and clinically important information up to three years post-treatment for the management of patients with advanced liver disease and DAA-induced SVR. Nevertheless, conclusions should be drawn cautiously and with consideration of some limitations. First, the mode and duration of antiviral therapy were not controlled by the study protocol. Second, heterogeneity of the study cohort has to be taken into account, as patients with and without HIV coinfection as well as patients with and without concomitant HCC had been included. Third, assessments of fibrosis and portal hypertension were done by non-invasive techniques, exclusively. While it would have been desirable to assess liver fibrosis by repeated liver biopsies and portal hypertension by repeated measurement of hepatic venous pressure gradient, these invasive procedures would have been risky in our patient collective and were not covered by the ethic committee vote.

In conclusion, our study shows that liver stiffness, but not spleen stiffness, continues to decline in a considerable proportion of patients with liver cirrhosis after HCV eradication had been achieved. Therefore, all patients with advanced liver disease should be offered a surveillance programme for complications of portal hypertension despite HCV eradication and regression of liver stiffness.

CONFLICT OF INTEREST

The authors who have taken part in this study declare that they do not have anything to disclosure regarding conflict of interest with respect to the manuscript.

DATA AVAILABILITY STATEMENT

The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

ORCID

Viola Knop https://orcid.org/0000-0002-7434-5218

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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