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Rapid liver and spleen stiffness improvement in compensated advanced chronic liver disease patients treated with oral antivirals

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Abstract

Background: We aimed to investigate the early changes in liver and spleen stiffness measurement (LSM, SSM) in hepatitis C virus (HCV) patients with compensated advanced chronic liver disease (cACLD) treated with new antivirals (DAA) to elucidate factors determining the initial change in stiffness and its implications for the long-term follow up of HCV-cured patients.

Methods: A total of 41 patients with cACLD who started DAA therapy underwent LSM and SSM at baseline, week 4, end of treatment (EOT), 24 and 48 weeks of follow up using transient elastography.

Results: LSM improved rapidly during the first 4 weeks of treatment (baseline: 20.8kPa; week 4: 17.5kPa, $p = 0.002$), with no significant changes between week 4 and EOT (18.3kPa, $p = 0.444$) and between EOT and 48-week follow up (14.3kPa, $p = 0.148$). Likewise, SSM improved rapidly (baseline: 45.7kPa; week 4: 33.8kPa, $p = 0.047$), with no significant changes between week 4 and EOT (30.8kPa, $p = 0.153$) and between EOT and 48-week follow up (31.2kPa, $p = 0.317$). A higher decrease in LSM was observed in patients with baseline ALT $\geqslant$ twofold upper limit normal ($2 \times$ ULN) than in those with ALT $< 2 \times$ ULN (–5.7kPa *versus* –1.6kPa). Patients who presented a decrease in LSM $\geqslant 10\%$ during treatment compared with those with LSM $< 10\%$ decrease, showed lower SSM values, higher platelet counts and lower bilirubin levels at 24-week follow up. Those with decrease in SSM $\geqslant 10\%$, presented a higher increase in platelets than those with SSM $< 10\%$ change ($p = 0.015$).

Conclusions: LSM and SSM decrease very rapidly during DAA treatment in cACLD patients suggesting that it most probably reflects a reduction in inflammation rather than in fibrosis. cACLD patients should be maintained under surveillance independently of stiffness changes, because advanced fibrosis can still be present.

Keywords: compensated advanced chronic liver disease (cACLD), direct-acting antiviral agents (DAA), hepatitis C, inflammation, liver stiffness, spleen stiffness

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Introduction

The immediate goal of hepatitis C virus (HCV) treatment is to achieve sustained virological response (SVR), but the ultimate aim of HCV eradication is trying to improve long-term outcomes (progression of fibrosis, clinical decompensation and possibly death). Several studies have demonstrated that in patients with SVR after interferon-based treatment, liver fibrosis can regress during follow up.[1,2] Moreover, in those patients who achieve SVR, a decrease in hepatic venous pressure gradient (HVPG) can

occur and, therefore, a decrease in complications associated to portal hypertension is observed.[3–5] A lower incidence of hepatocellular carcinoma[6,7] and a reduction of all-cause mortality have also been documented.[8]

Until 2014, interferon-based treatment was the standard of care for HCV therapy. This treatment was not free from complications, especially in patients with advanced fibrosis and cirrhosis, in whom low rates of SVR were observed.[9–11] However, the introduction of new direct-acting antiviral agents (DAA) in the current treatment for HCV patients have changed this paradigm, achieving high SVR rates with minimal side effects, even in patients with cirrhosis.[12] In consequence, a huge number of patients with advanced chronic liver disease are achieving SVR, highlighting the importance of monitoring fibrosis regression to identify those patients with a higher risk of developing complications during follow up. Non-invasive methods such as transient elastography (TE) may be useful for monitoring these changes. Liver stiffness has been correlated with degree of liver fibrosis, the presence of clinically significant portal hypertension and with the risk of decompensation.[13–15] On the other hand, recently, spleen stiffness has been shown to be also correlated with HVPG and the risk of decompensation.[16,17] However, it has to be taken into account that non-invasive methods to evaluate liver fibrosis have not been validated in non-viremic patients and changes in liver stiffness after a successful antiviral treatment might not accurately reflect a real change in residual fibrosis, but rather a reduction in inflammation.

The aim of our study was to investigate the early changes in liver and spleen stiffness measurement (LSM, SSM) in HCV patients with compensated advanced chronic liver disease (cACLD) treated with new oral DAA in order to elucidate the factors determining the initial changes in stiffness and their implications for the long-term follow up of HCV-cured patients.

Patients and methods

Consecutive compensated patients with baseline LSM $\geqslant$10 kPa who met the Baveno VI criteria for cACLD[18] and in whom treatment with oral DAA was approved were included in this prospective small-scale study. As per definition, patients with LSM $\geqslant$15 kPa were considered highly suggestive of having cACLD and, for patients with LSM between 10 and 15 kPa, one of the following criteria was needed to confirm cACLD: platelet count $<150 \times 10^9$/l, spleen size $\geqslant$13 cm, nodular liver or collateral circulation in abdominal ultrasound, HVPG >5 mmHg, upper gastrointestinal endoscopy showing gastroesophageal varices or previous liver biopsy showing bridging fibrosis or cirrhosis. Patients with previous decompensation were excluded. The recruitment period started in January 2015 (date of the beginning of widespread DAA therapy in Spain) and finished in June 2015.

We calculated the sample size, taking into account improvement in LSM [defined as a 10% decrease in LSM from baseline to end of treatment (EOT)] could be found in 60% of treated patients with a total width of confidence interval (CI) of 30% (45–75%), that is more than four times the background 10% improvement in untreated patients. Aiming at a confidence level of 95%, a normal approximation to the binomial calculation would require a total sample size of 41 patients.[19]

The study [ClinicalTrials.gov identifier NCT 02439567] was registered on 27 April 2015.

Patients received a treatment regimen adequate for their HCV genotype. Patients with genotype 1 or 4 received treatment with Sofosbuvir 400 mg daily (Sovaldi, Gilead, Cambridge, UK), Simeprevir 150 mg daily (Olysio, Jansen, Beerse, Belgium) and weight-based dose of ribavirin (RBV) (with ranging dose 800–1200 mg) for 12 weeks. For genotype 3, patients received Sofosbuvir 400 mg daily, Daclatasvir 60 mg daily (Daklinza, Bristol-Myers Squibb, Uxbridge, UK) and a weight-based dose of RBV for 24 weeks. No other genotypes were found in our sample.

All patients underwent LSM and SSM, and biochemical tests at baseline, week 4, EOT and at 24 and 48 weeks of follow up after finishing treatment. SVR was defined as undetectable HCV-RNA at 12 weeks follow up after finishing treatment. An abdominal ultrasound was performed at baseline and every 6 months, as part of standard routine surveillance for hepatocellular carcinoma.

The study was approved by the Ethics Committee of Hospital Universitari Vall d'Hebron (CEIC) (JOA-SOF-2015-01) and was conducted in accordance with the 1975 Declaration of Helsinki

and Good Clinical Practice guidelines. All patients gave written informed consent before the inclusion.

Hepatitis C virus–ribonucleic acid quantification

Serum HCV-RNA was tested at baseline, during treatment (weeks 4, 12 or 24) and 12 weeks after treatment completion. A real-time polymerase chain reaction-based test (Cobas Ampliprep/Cobas TaqMan; Roche Molecular Diagnostics, West Sussex, UK; detection limit 15 IU/ml) was used for HCV detection and quantization. HCV genotyping was performed by deep sequencing on a 454/GS-Junior (Roche, Branford, CA, USA) platform.

Liver stiffness measurement

LSMs by TE (Fibroscan® 502 Touch, Echosens, Paris, France) were performed by a single operator with experience in more than 500 procedures (MP). LSMs were performed in a fasting state according to the usual standard procedure. Only LSMs with success rate of $\geqslant$60% (with at least 10 valid measurements) and an interquartile-range-to-median-LSM ratio of $\leqslant$30% were selected as valid measures. Medium or extra-large probes were selected as per device indication and, for each patient, the same probe was used during all study visits.

Spleen stiffness measurement

SSMs were performed with TE using Fibroscan® 502 Touch at the same appointment, with the same probe and the same software used for LSM, with the patient in the supine position and the left arm in maximal abduction. The spleen was localized under ultrasound assistance (Vscan®, General Electric Healthcare, Milwaukee, WI, USA), and the probe was positioned where the spleen was correctly visualized. Reliable results for spleen stiffness have not been yet validated. Therefore, the same reliable criteria for the LSM were applied.

Statistical analysis

Categorical variables are expressed as numbers (percentages) and continuous variables as median (25th percentile–75th percentile). For statistical analyses and presentation of results, differences between categorical variables were assessed by Chi-square test or Fisher's exact test, when necessary. Continuous variables were compared using the Student's *t* test or Mann–Whitney test as appropriate. Intragroup comparisons were made using Wilcoxon's test for paired data. The general linear model technique for analysing repeated measures was used to examine changes in biochemical parameters and TE over time. *p* values below 0.05 were considered statistically significant and, in paired-sample comparisons, the Bonferroni correction was applied. Statistical analyses were performed using SPSS v. 19.0 software (IBM, Armonk, NY, US) and STATA 13.1 statistical software (StataCorp, College Station, TX, US).

Table 1. Baseline characteristics of the patients included.

Characteristics	Patients $n = 41$
Male sex, *n* (%)	20 (48.8)
Age, years	68 (59–75)
BMI, kg/m^2	26.6 (24.9–29.4)
Ethnicity, *n* (%)	
White	41 (100)
HCV genotype, *n* (%)	
1–4	39 (95.1)
3	2 (4.9)*
Treatment naïve, *n* (%)	18 (43.9)
Spleen size, cm	12.9 (11.5–13.7)
Varices $n = 31$, *n* (%)	
No/I/II-III	15 (48.4)/14 (45.2)/2 (6.5)
HCV RNA level, $\log_{10}$ IU/ml	6.3 (6.0–6.6)
Liver stiffness, kPa	20.8 (16.3–29.5)
Spleen stiffness, kPa	45.7 (26.6–65.2)
Platelets, 10^9/l	106.5 (82–142.5)
ALT, IU/l	78 (55–135)
ALT $\geqslant 2 \times$ ULN, *n* (%)	17 (41.5)
Bilirubin, mg/dl	0.89 (0.68–1.11)
Albumin, g/dl	3.84 (3.64–4.12)

*One patient had a mix of genotype 1 and 3. Continuous values expressed as median (25th percentile –75th percentile). BMI, body mass index; HCV, hepatitis C virus; ALT, alanine aminotransferase; $2 \times$ ULN, twofold upper limit normal.

Results

The baseline characteristics of the 41 patients with cACLD included are described in Table 1. All

Table 2. Laboratory parameters at study time points.

	Baseline	Week 4	EOT	24-week FU*	*p* value
Haemoglobin (g/dl)	14.4 (12.9–15.7)	12.1 (11.4–13.2)	11.8 (11–13.0)	14 (13–15.6)	<0.001
Platelets ($\times 10^9$/l)	106.5 (82–142.5)	139.5 (107.5–166.5)	135.5 (106.5–171.5)	122 (104–161)	<0.001
Bilirubin (mg/dl)	0.89 (0.68–1.11)	1.41 (1.05–2.5)	1.16 (0.91–1.8)	0.73 (0.57–0.86)	<0.001
ALT (UI/l)	78 (55–135)	18 (15–21)	16 (15–20)	18 (16–23)	<0.001
AST (UI/l)	92 (66–129)	26 (23–32)	27 (23–30)	26 (23–32)	<0.001
GGT (UI/l)	92 (61–131)	38 (31–50)	28 (22–37)	35 (24–52)	<0.001
Albumin (g/dl)	3.84 (3.64–4.12)	4.04 (3.65–4.20)	4 (3.60–4.28)	4.20 (3.90–4.50)	<0.001

*Data of 48-week follow up were equal to 24-week follow up therefore, to simplify the table, they are not shown in the table. Values expressed as median (25th percentile–75th percentile). ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; EOT, end of treatment; FU, follow up.

patients were in Child-Pugh class A. Thirty-six patients (87.8%) were infected by genotype 1, and three patients (7.3%) were infected by genotype 4. Two patients had genotype 3 (one of them with a mixed genotype 1 and 3) and both were treated with Sofosbuvir + Daclatasvir + RBV for 24 weeks. Seventeen patients (41.5%) presented with alanine aminotransferase (ALT) levels higher than twofold upper limit normal (2 × ULN) at baseline and most of them were male (12 men *versus* 5 women).

Changes in laboratory parameters

At week 4, all patients presented with undetectable HCV-RNA. SVR was observed in 40 patients (97.6%). The patient who did not achieve SVR relapsed after finishing treatment and was HCV-genotype 1b.

Biochemical parameters improved rapidly after starting treatment (Table 2), except for haemoglobin and bilirubin that were altered during treatment due to RBV, but returned to baseline after finishing treatment.

Changes in liver and spleen stiffness during treatment

Figure 1 shows changes in liver stiffness measurements (LSM) and spleen stiffness measurements (SSM) during the study period. All patients had reliable LSM. Nine patients (22%) were not included in pairwise comparisons of SSM. One of them due to a previous splenectomy and the others due to unreliable results at some study point.

For the patient who did not achieve SVR, LSM and SSM were no longer performed after finishing treatment. LSM and SSM for this patient during treatment are represented in supplementary Figure 1.

Globally, LSM during treatment improved. Median LSM values were: 20.8 kPa (16.3–29.5 kPa) at baseline, 17.5 kPa (13.5–26.3 kPa) at week 4 and 18.3 kPa (13.3–27.2 kPa) at the EOT ($p = 0.014$). LSM improved rapidly and significantly during the first 4 weeks of treatment ($p = 0.002$), with no significant changes between week 4 and EOT ($p = 0.444$). The median change from baseline to week 4 was –4.8 kPa (95% CI: –6.4 kPa to –1.0 kPa) and from baseline to EOT was –3.3 kPa (95% CI: –5.9 to –0.5 kPa) (Figure 1A).

Significant changes in SSM were observed during treatment. Median SSM was 45.7 kPa (26.6–65.2 kPa) at baseline, 33.8 kPa (26.3–46.4 kPa) at week 4 and 30.8 kPa (21.3–39.1 kPa) at the EOT ($p = 0.012$). Similarly to LSM, SSM improved rapidly and significantly during the first 4 weeks of therapy ($p = 0.047$), with no significant changes between week 4 and EOT ($p = 0.153$). The median change from baseline to week 4 was –5.7 kPa (95% CI: –11.4 kPa–0 kPa) and from baseline to EOT –6.6 kPa (95% CI: –12.8 kPa to –1.8 kPa) (Figure 1B).

Based on ALT levels, median basal LSM was 20.8 kPa in both groups (baseline ALT $\geq 2 \times$ ULN and baseline ALT $< 2 \times$ ULN), however, patients who had baseline ALT $\geq 2 \times$ ULN presented a higher decrease in LSM during treatment, median –5.7 kPa (95% CI: –9.7–0.2 kPa), than patients with baseline ALT $< 2 \times$ ULN, median –1.6 kPa (95% CI: –5.2–2.7 kPa) ($p = 0.037$) (Figure 2).

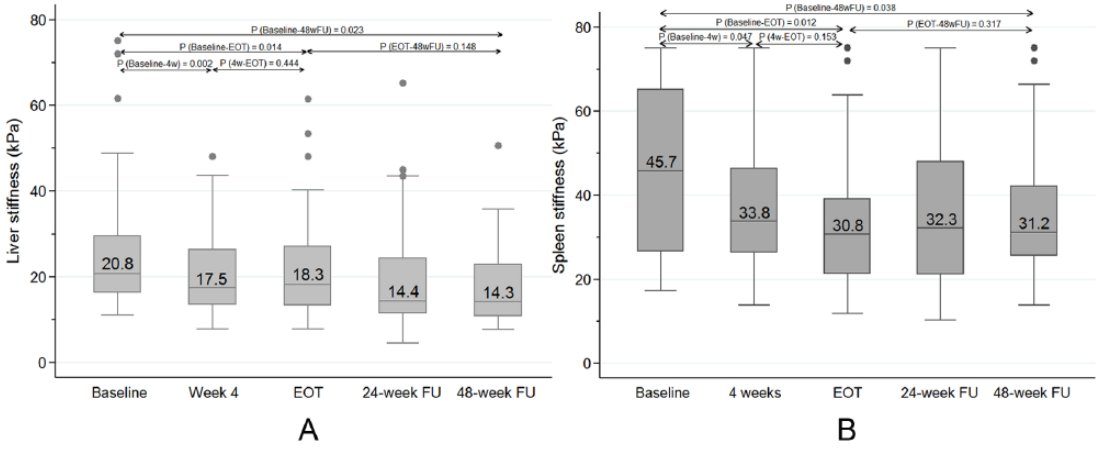

Figure 1. (A) Liver stiffness measurement at baseline, week 4, end of treatment (EOT), 24- and 48-week follow up (FU) in the 40 hepatitis C compensated advanced chronic liver disease (cACLD) patients cured with therapy. (B) Spleen stiffness measurement (SSM) at baseline, week 4, EOT, 24- and 48-week FU in the 32 hepatitis C cACLD patients with reliable values of SSM.
EOT, end of treatment; FU, follow up.

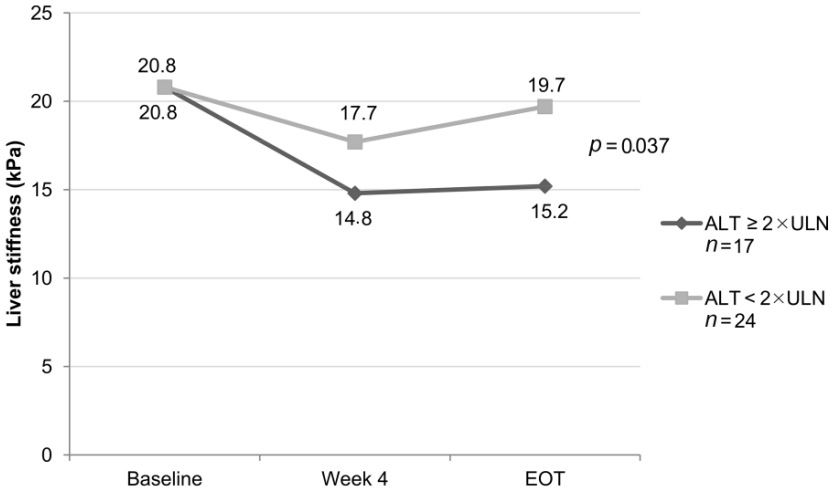

Figure 2. Median liver stiffness measurement (LSM) during treatment of hepatitis C virus compensated advanced chronic liver disease patients based on baseline (pretreatment) alanine aminotransferase (ALT) levels. Patients with baseline ALT ⩾ 2 × ULN presented a higher decrease in LSM.
cACLD, compensated advanced chronic liver disease; HCV, hepatitis C virus; ALT, alanine aminotransferase; 2 × ULN, twofold upper limit normal; EOT, end of treatment.

Changes in liver and spleen stiffness after treatment

As shown in Figure 1A, during follow up, from EOT to week 48, LSM continued improving, especially until week 24, although this improvement was not statistically significant ($p = 0.148$). Median LSM at 24 weeks of follow up (24w-FU) was 14.4 kPa (11.5–26.3 kPa) and at 48 weeks of follow up (48w-FU) was 14.3 kPa (10.8–22.9 kPa). On the other hand, SSM remained stable from EOT to 48w-FU (Figure 1B). Median SSM was 32.3 kPa (21.5–46.4 kPa) at 24w-FU and 31.2 kPa (25.5–42.2 kPa) at 48w-FU. However, both LSM and SSM decreased significantly from

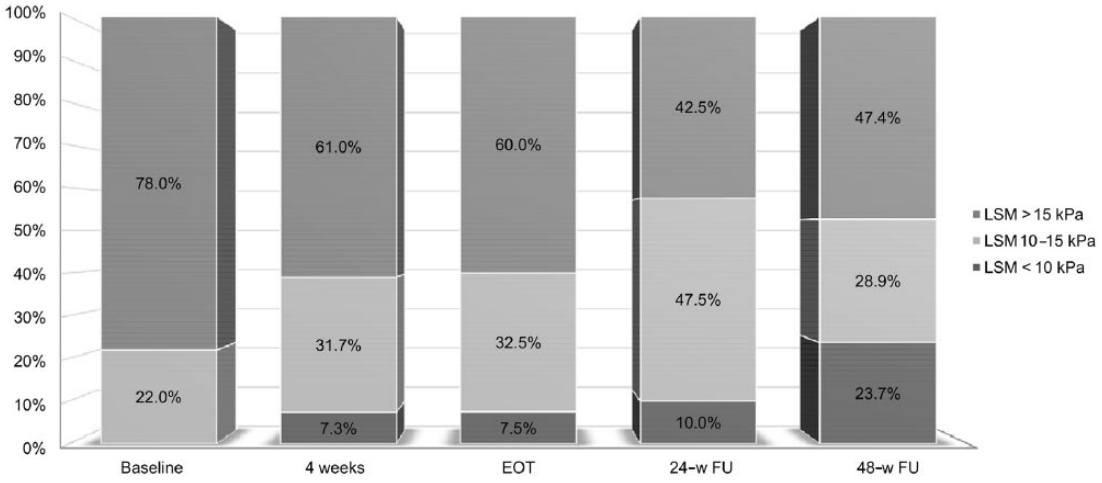

Figure 3. Proportion of treated hepatitis C virus compensated advanced chronic liver disease patients with liver stiffness measurement (LSM) < 10 kPa, 10–15 kPa and >15 kPa at each study time point.
cACLD, compensated advanced chronic liver disease; HCV, hepatitis C virus; LSM, liver stiffness measurement.

baseline to end of follow up, with a median change in LSM of –5.5 kPa (–7.4 to −2.7 kPa, $p = 0.023$) and median change in SSM of –7.1 kPa (–9.6 to −0.3 kPa, $p = 0.038$).

Figure 3 shows that the proportion of patients with LSM >15 kPa decreased progressively from the beginning of treatment until the end of follow up ($p = 0.003$), while 24% of the study population had their LSM reduced below the 10 kPa threshold.

Comparison of patient characteristics according to change in liver and spleen stiffness

Patients who presented a decrease in LSM ⩾ 10% during treatment, calculated as (EOT LSM–baseline LSM)/baseline LSM ×100, were considered to present a significant LSM improvement. Twenty-three patients (57.5%) had a significant LSM improvement. Differences between patients with or without significant LSM improvement are described in Table 3. As seen, patients with significant LSM improvement showed lower SSM values, higher platelet counts and lower bilirubin levels at 24w-FU. In addition, those patients with significant LSM improvement presented a higher decrease in SSM during treatment ($p = 0.027$) than patients without significant LSM improvement ($p = 0.870$) (Figure 4).

Platelet count was also correlated with changes in SSM. Patients with improvement in SSM ⩾ 10% (18 patients, 56.3%) from baseline to EOT, presented a higher increase in platelets, compared with those who did not present an improvement in SSM ($p = 0.015$) (Figure 5).

Liver stiffness measurement and spleen stiffness measurement changes according to surrogate markers of portal hypertension

Changes in LSM and SSM were also analysed in patients with different degrees of portal hypertension. Since HVPG measurements were not available and endoscopies were not performed in all patients, platelet count (<150 × 10^9/l or ⩾150 × 10^9/l) and LSM (<20 kPa or ⩾20 kPa) were used as surrogate markers of portal hypertension. As seen in supplementary Figure 2, patients with normal or low platelets presented similar changes in LSM, while significant LSM decrease was only observed in patients with basal LSM ⩾ 20 kPa. By contrast, significant SSM changes were only observed in patients with platelet counts < 150 × 10^9/l or LSM ⩾ 20 kPa, probably patients with higher basal portal pressure. Patients with low (normal) SSM and probably lower portal pressure remained unchanged.

Discussion

Treatment of HCV with the new DAA is extremely effective, causing a very rapid control of viral replication, normalization of transaminases and disappearance of liver inflammation. Consequently, the virological and biochemical profile of these new treatments is surely very different from the prior interferon-based therapies, in which a slower and longer process was

Table 3. Main characteristics in patients who presented a decrease in liver stiffness measurement (LSM) during treatment ⩾ 10% compared with those who presented a decrease in LSM <10%. Change in LSM is calculated as: (end-of-treatment LSM-Baseline LSM)/Baseline LSM ×100.

Characteristics	LSM ⩾ 10% (*n* = 23)	LSM < 10% (*n* = 17)	*p* value
Male sex, *n* (%)	13 (56.5)	7 (41.2)	0.491
Age, years	68 (63–75)	67 (58–73)	0.448
BMI, kg/m^2	26.0 (24.2–27.9)	27.4 (25.2–30.0)	0.251
Treatment-naïve, *n* (%)	10 (43.5)	8 (47.1)	0.822
Spleen size, cm	12.5 (11–13.6)	12.8 (11.9–13.5)	0.133
Baseline HCV RNA level, $\log_{10}$ IU/ml	6.5 (6.2–6.6)	6.2 (5.9–6.4)	0.085
Liver stiffness, kPa			
Baseline	20.8 (17.3–35.3)	17.5 (14.1–29.5)	0.163
24-week FU	12.8 (11.1–26.3)	14.5 (11.7–24.3)	0.487
Spleen stiffness, kPa			
Baseline	45.7 (28–65.2)	42.9 (26.5–65.1)	0.907
24-week FU	29.9 (21.5–37.4)	39.5 (20.4–59.4)	0.046
Haemoglobin, g/dl			
Baseline	14.5 (13.3–15.6)	14.2 (12.6–15.8)	0.389
EOT	11.4 (11–12.9)	11.8 (11.2–13)	0.976
Platelets, 10^9/l			
Baseline	109 (84–144)	104 (79–134)	0.571
24-week FU	133 (117–157)	105.5 (91.5–168.5)	0.054
ALT, IU/l			
Baseline	94 (53–135)	69 (55–131)	0.681
24-week FU	18.5 (16–25)	16.5 (16–22.5)	0.398
Bilirubin, mg/dl			
Baseline	0.72 (0.55–0.96)	0.93 (0.86–1.24)	0.013
24-week FU	0.59 (0.48–0.72)	0.83 (0.77–1.11)	<0.001
Albumin, g/dl			
Baseline	3.8 (3.7–4.1)	3.9 (3.5–4.1)	0.847
24-week FU	4.3 (4–4.6)	4.2 (3.9–4.5)	0.362

Continuous values expressed as median (25th percentile–75th percentile). HCV, hepatitis C virus; ALT, alanine aminotransferase; FU, follow up; BMI, body mass index; LSM, liver stiffness measurement.

probably taking place. This rapid on–off response with DAA becomes a very interesting model for investigating the dynamics of liver and spleen stiffness and learn about the contribution of the different components causing increased tissue stiffness. The results of the present study clearly indicate that liver and spleen stiffness improve very early during treatment, as early as at 4 weeks of therapy or even before, and more importantly, that this initial decrease explains for most of the final liver and spleen stiffness observed for the first 48 weeks of post-treatment follow up.

Previous studies have shown improvement in liver stiffness during long-term follow up, especially in patients who achieve SVR treated with

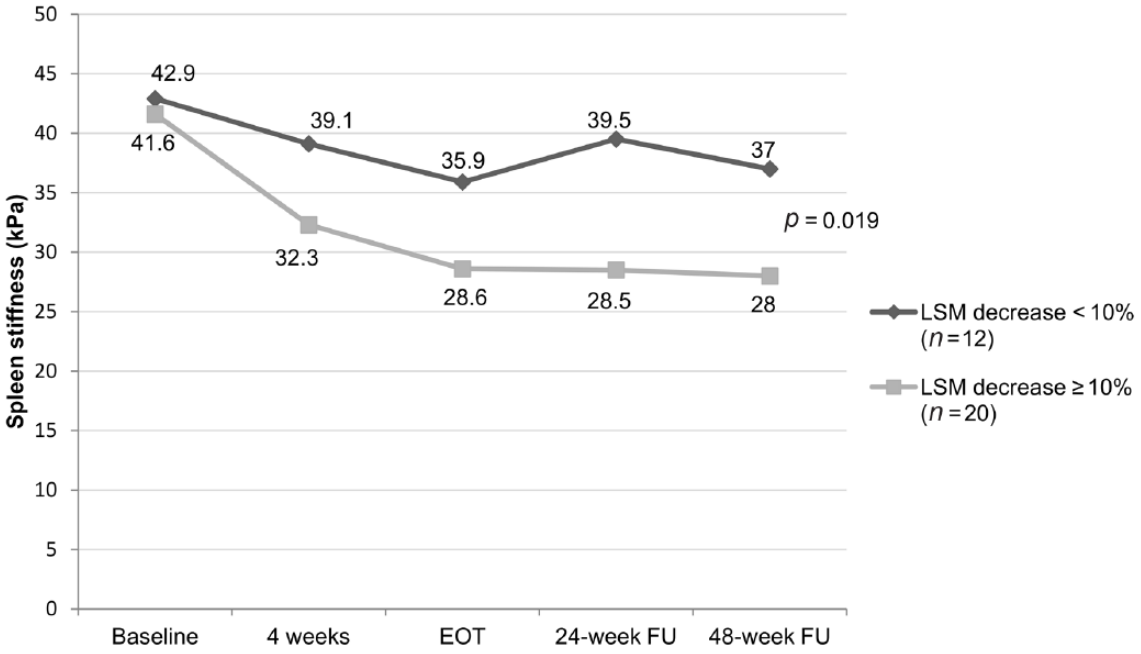

Figure 4. Spleen stiffness measurement (SSM) changes during treatment comparing hepatitis C virus compensated advanced chronic liver disease patients with a significant improvement in liver stiffness measurement (LSM) during treatment (decrease in LSM ⩾ 10% from baseline to EOT) with patients without a significant improvement (decrease in LSM < 10%). Only patients with all SSM reliable measures are represented.
cACLD, compensated advanced chronic liver disease; HCV, hepatitis C virus; FU, follow up; EOT, end of treatment; LSM, liver stiffness measurement.

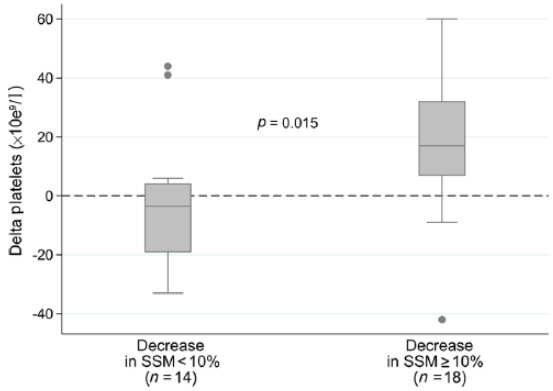

Figure 5. Change in platelets in treated hepatitis C virus compensated advanced chronic liver disease patients from baseline (pretreatment) to 24-week follow up, based on decrease in spleen stiffness measurement from baseline to EOT. Delta platelets = 24-week follow up platelet count–baseline platelet count.
cACLD, compensated advanced chronic liver disease; HCV, hepatitis C virus; SSM, spleen stiffness measurement.

interferon-based therapies. Evaluation of LSM at week 4 of treatment was rarely performed. Hézode *et al.*[20] studied patients treated with pegylated interferon-α plus RBV, and they found that patients with cirrhosis who achieved SVR presented a median decrease in LSM of 4.1 kPa at week 4 compared with a decrease of 0.7 kPa in patients with no cirrhosis. By contrast, Bernuth *et al.*[21] found that in patients with chronic HCV infection receiving sofosbuvir-based treatment, LSM increased at week 4, from a baseline LSM of 8 kPa to 12.9 kPa: one of the reasons given by the authors to explain these results is that patients did not fast prior to TE and also that the anaemia caused by combined therapy might have increased liver blood flow and liver stiffness.

One of the most remarkable findings of the present study is that the rapid liver and spleen stiffness improvement observed at 4 weeks of therapy explains most of the decrease in stiffness observed during the total 15 months of follow up. Indeed, although there was a small decline in LSM from week 4 to end of follow up (median change –1.5 kPa), approximately 75% of the decrease was observed during the first 4 weeks of therapy (median change –4.8 kPa). This observation, together with the significant correlation between

high ALT at baseline and higher decrease in LSM at 4 weeks, suggests that the main driver for the liver stiffness improvement is suppression of liver inflammation, as a consequence of viral eradication rather than a pure reduction of liver fibrosis. This conclusion bears several implications. First, that in patients with HCV cACLD, 15–20% of the observed liver stiffness is probably due to inflammation. In this regard, inflammation-adapted LSM cut-offs have been proposed, but there is a controversy about its usefulness.[22,23] Second, this explains in great part the observed discrepancies between liver histology and liver stiffness after HCV eradication. As seen in our patients, significant changes in LSM occur in 4 weeks, while it has been well documented that detectable changes in fibrosis require much more time. In our sample, 78% of our patients had baseline LSM $\geqslant$ 15 kPa and at the end of follow up, this percentage was reduced to 47%, while the percentage of patients with LSM $<$ 10 kPa and with LSM between 10–15 kPa increased progressively. However, D'Ambrosio *et al.*[24] demonstrated that 21% of patients with LSM $<$ 12 kPa after an average of 61 months from SVR still had cirrhosis in liver biopsy, indicating less accuracy of TE for diagnosing cirrhosis in nonviremic patients. Thus, in order to validate the clinical meaning of post-treatment LSM values, longer follow-up studies will be needed, taking into consideration the information regarding basal LSM, changes in LSM during follow up and post-therapy time frames.

In addition to LSM improvement, SSM also remarkably improved in a similar (and even more pronounced) pattern. All SSM improvement was seen during the first 4 weeks of therapy, with no additional change for the rest of the follow up. Again, this rapid decrease, along with the strong correlation with the decrease in liver stiffness (and liver inflammation), suggests that the main driver for this improvement is not only spleen congestion and portal hypertension decrease. Although spleen stiffness has been correlated with HVPG[16,25,26] and a reduction in HVPG during HCV treatment has been demonstrated in previous studies,[3,4,27] it seems plausible that other explanations are needed for this rapid change in spleen stiffness. In that sense, the splenomegaly classically associated to portal hypertension could be considered as a composite of congestion, enlargement and hyperplasia of splenic lymphoid tissue (white pulp), and increased angiogenesis and fibrogenesis.[28,29] In addition, increased splenic inflammation might have an additional role in HCV-infected patients, considering that spleen could be regarded as a large lymph node and HCV-infected patients consistently show hepatic perihiliar adenomegalies on liver imaging. Altogether, these findings point out to a rapid improvement in spleen inflammation or spleen remodelling due to a decrease in lymphoid tissue infiltration as the main cause for the initial spleen stiffness decrease. However, a very early decrease in portal pressure due to reduced liver inflammation could also partially contribute to decrease spleen stiffness.

Finally, as expected, all analytical parameters improved during follow up, and those not affected by RBV, improved also very early (4 weeks) during therapy. Platelet counts also followed this pattern, and again, most of the improvement observed during follow up occurred at 4 weeks of therapy. Remarkably, patients in whom a significant improvement of SSM was observed presented the greater increase in platelet counts, as compared with patients without significant SSM improvement (Fig. 5). Similar results were obtained comparing patients with or without LSM improvement. Although a correlation between changes in spleen size and increase in platelet counts after HCV therapy has been reported,[30] in our case, differences in spleen size were not observed, probably due to the short follow up. Platelet counts have been shown to increase years after SVR due to improvement in thrombopoietin production, improvement in portal hypertension and reversal of splenomegaly.[30] In our case, the rapid improvement and correlation with SSM changes suggests that platelets increase mainly due to spleen release secondary to decreased spleen sequestration.

The limitations of the present study include the small sample size and the lack of external validation. The small sample size limits the ability to control confounding factors through multivariate regression analysis. Also the lack of simultaneous liver biopsy and HVPG information is a weakness of our study with a very difficult solution, since it would be probably unethical to perform them at 4 weeks of therapy. Moreover, SSM may be technically difficult to perform in clinical practice due to the need to carry out an ultrasound prior to TE to localize the spleen, and the fact that TE is not optimized for SSM. As a consequence of that, not all patients could be evaluated for SSM and the

results might have been altered. However, we feel that this is not the case, since SSM correlated very well with LSM and platelet findings, and SSM values remained very constant after the initial decrease. Another limitation is that most of the patients (88%) were genotype 1 and all of them were White. Both genotype and ethnicity have been known to affect kinetics of liver fibrosis related to HCV.[31] Finally, the lack of a longer follow up limits the capacity to detect long-term changes in LSM and SSM that are probably related to fibrosis improvement.

In conclusion, liver and spleen stiffness decrease significantly and very rapidly during DAA treatment of HCV-infected cACLD patients and this improvement accounts for most of the stiffness improvement observed during follow up, suggesting that it most probably reflects liver and spleen improvement in inflammation and cell infiltration. These findings have important clinical implications for the follow up of cACLD HCV-cured patients, since changes in LSM after SVR cannot be interpreted just as a reduction of liver fibrosis (at least during the first year of follow up). Consequently, patients with cACLD prior to SVR cannot be discharged from follow up based on LSM improvements. Until more information from patients with longer follow up and with liver biopsy information is gathered, patients will have to remain under surveillance.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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