Received: 7 October 2020 | Revised: 23 August 2021 | Accepted: 1 September 2021

DOI: 10.1111/liv.15058

Liver INTERNATIONAL WILEY

ORIGINAL ARTICLE

Elastography is unable to exclude cirrhosis after sustained virological response in HCV-infected patients with advanced chronic liver disease

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Abstract

Background: Liver fibrosis and transient elastography (TE) correlation in hepatitis C virus (HCV)-infected patients with compensated advanced chronic liver disease (cACLD) after the sustained virological response (SVR) is unknown.

Aims: To evaluate TE accuracy at identifying cirrhosis 3 years after HCV-eradication.

Methods: Prospective, multi-centric study including HCV-cACLD patients before direct-acting antivirals (DAA). Diagnostic accuracy of TE (area under ROC, AUROC) to identify cirrhosis 3 years after SVR was evaluated.

Results: Among 746 HCV-infected patients (95.4% with TE ≥10 kPa), 76 (10.2%) underwent a liver biopsy 3 years after SVR. Before treatment, 46 (63%) showed a TE>15 kPa. The TE before DAA was the best variable for predicting cirrhosis (METAVIR, F4) after SVR (AUROC = 0.79). Liver function parameters, serological non-invasive tests (APRI and FIB-4), and TE values improved after SVR. However, liver biopsy 3 years after HCV elimination (median time = 38.4 months) showed cirrhosis in 41 (53.9%). Multivariate analysis (OR (95% CI), *P*) showed that HCV-genotype 3 (20.81 (2.12-201.47), .009), and TE before treatment (1.21 (1.09-1.34), <.001) were the only variables associated with cirrhosis after SVR. However, the accuracy of TE after SVR was poor (AUROC = 0.75) and 6 (27.3%) out of 22 patients with a TE <8 kPa had cirrhosis. Similar results were found with APRI and FIB-4 scores.

Conclusions: Cirrhosis is present, 3 years after SVR, in more than half of HCV-cACLD patients even with the normalisation of liver function parameters, serological non-invasive tests and TE values. The low diagnostic accuracy of non-invasive methods after SVR reinforces the need for long-term surveillance.

Abbreviations: ALT, alanine aminotransferase; APRI, AST to platelet ratio; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristics; BMI, body mass index; cACLD, compensated advanced chronic liver disease; CSPH, clinically significant portal hypertension, N, number; DAA, direct-acting antiviral; EoT, end of treatment; FIB-4, fibrosis-4 index; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; INR, international normalised ratio; IQR, interquartile range; MELD, model for end-stage liver disease; NASH CRN, NASH clinical research network; NITs, serological non-invasive tests; NPV, negative predictive value; PLT, platelet; PPV, positive predictive value; SAEs, serious adverse events; SVR, sustained virological response; T2DM, type 2 diabetes mellitus; TE, transient elastography; γGT, gamma-glutamyl transpeptidase.

Teresa Broquetas and Paula Herruzo-Pino collaborated equally

The corresponding author of this manuscript certifies that the contributors' and conflicts of interest statements included in this paper are correct and have been approved by all co-authors.

Funding information
None.

Handling Editor: Alessio Aghemo

KEYWORDS
cirrhosis, fibrosis, hepatitis C, liver biopsy, sustained virological response, transient elastography

1 | INTRODUCTION

Hepatitis C virus (HCV) infection is a major public health problem affecting over 71 million people worldwide, and it is responsible for more than 399 000 deaths from liver cancer and cirrhosis yearly.[1]

The evaluation of liver fibrosis in patients with chronic HCV infection is crucial, since the late stages of the disease are related to poorer prognosis.[2] Patients with cirrhosis can develop portal hypertension, oesophageal varices, hepatocellular carcinoma (HCC), and liver failure. Therefore, they require stricter follow-up, with abdominal ultrasound every six months and upper gastrointestinal endoscopies every 2 years.[2,3] Liver biopsy still remains the gold standard for fibrosis stage assessment,[2] with multiple staging systems, such as the METAVIR[4] and Laennec[5] scales. Since liver biopsy is an invasive method implying risks, the current guidelines recommend that HCV-infected patients with compensated advanced chronic liver disease (cACLD) should initially be assessed and classified using non-invasive methods.[6] Both, transient elastography (TE) that assesses liver stiffness, and serological non-invasive tests (NITs) as AST to platelet ratio (APRI) and fibrosis-4 index (FIB-4), have been shown to be useful at identifying patients with cirrhosis before antiviral treatment.[3,7] The TE is the simplest, best evaluated and most widely used elastographic technique and it is considered the standard non-invasive method in clinical practice. It offers an excellent correlation with liver fibrosis, well-established quality criteria and a high diagnostic reliability for identifying patients with cirrhosis.[4,8] Therefore, a TE cut-off value of 14 kPa identifies and excludes early cirrhosis with a positive predictive value (PPV) of 80%-90% and negative predictive value (NPV) of 94%-96% respectively.[9,10] However, the diagnostic accuracy of TE and NITs to predict residual fibrosis decreased in cirrhotic patients after the sustained virological response (SVR) to interferon.[11,12]

Direct-acting antiviral (DAA) therapy achieves SVR rates of >95% with only 8-12 weeks of treatment.[3] The SVR is associated with a decrease in mortality and morbidity,[13] by reducing the risk of liver complications.[14] The HCV elimination normalises transaminases, resolves necroinflammation, and improves TE and NITs independently of baseline fibrosis.[11,12] However, in patients with cACLD or cirrhosis, liver remodelling has not been widely studied after virological cure[15] and many patients remain with portal hypertension,[16] risk of HCC[17] and oesophageal varices.[18] European Association for the Study of the Liver guidelines recommend that patients with suspected advanced fibrosis (METAVIR score F3-4),[19] cACLD or cirrhosis before antiviral treatment should continue to undergo abdominal ultrasounds every 6 months because they still remain at risk of developing HCC.[17] On the other hand, The American Gastroenterological Association Institute Guidelines on the Role of Elastography in the Evaluation of Liver Fibrosis hypothesise that a post-treatment TE cut-off of 9.5 kPa can rule out advanced liver fibrosis after SVR and allow these patients to be discharged, although the level of evidence of such recommendation is low.[7]

Key points

- Our study shows that neither transient elastography nor serological non-invasive tests as APRI or FIB-4 are reliable enough to accurately identify liver fibrosis stage after sustained virological response in patients with advanced chronic liver disease (cACLD).
- In spite of finding significant improvements in liver function parameters, NITs and TE values after HCV clearance in HCV-cACLD patients, cirrhosis and advanced fibrosis are present in 50% and 70%, respectively, after 3 years of viral elimination, being recommendable a continuous surveillance after sustained virological response.

The primary endpoint of our study was to evaluate TE and NITs accuracy at identifying cirrhosis 3 years after HCV elimination compared to liver biopsy. The secondary objectives were to assess the impact of HCV eradication, and the variables associated with cirrhosis 3 years after SVR.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

This is an open, multi-centric, prospective study concerning four public hospitals in Catalonia: Hospital del Mar (Barcelona), Hospital Clínic (Barcelona), Hospital Germans Trias-Pujol (Badalona) and Consorci Sanitari Parc Taulí (Sabadell).

Inclusion criteria were: age >18 years old, history of viremic HCV infection with a TE >9 kPa compatible with advanced fibrosis (METAVIR, F3-4)[9,10,19,20] prior to DAA treatment and having achieved SVR between July 2015 and July 2016. Patients were enrolled after 3 years from end of treatment (EoT) (from April 2018 to April 2019) and agreed to have a liver biopsy and a new TE evaluation.

Exclusion criteria were: absence of informed consent, no SVR, HBV-coinfection, TE value without quality criteria, clinical

decompensation, HCC or comorbidities limiting survival, large oesophageal varices, anticoagulant treatment or severe thrombopenia (platelet -PLT- count $<60 \times 10^9$/L), or less than 3 years of follow-up.

2.2 | Clinical variables and follow-up

Data were recorded at 3 different time-points: before DAA treatment (baseline), at SVR (12 or 24 weeks after the EoT) and 3 years after EoT. Variables evaluated at baseline and 3 years after EoT were anthropometric and demographic characteristics, concomitant medication, blood test, NITs (APRI and FIB-4); Child-Pugh and MELD (model for end-stage liver disease) scores; TE values; indirect data of portal hypertension (ultrasound or endoscopic) and Baveno VI criteria to avoid endoscopy.[6,18] Variables at SVR were TE and blood tests. Included patients did undergo 6-month abdominal ultrasounds, regular upper endoscopy (every 12-24 months) according to international recommendations[21] and Baveno criteria.[6,18] The liver biopsy was performed 3 years after EoT.

Patients with a TE > 15 kPa before antiviral treatment were considered patients highly suggestive of cACLD,[6] and those with a TE ≥14 kPa with a high suspicion of cirrhosis.[9,10] Patients were followed-up until 1 April 2020 or early discontinuation if voluntary withdrawal, de novo clinical decompensation, HCC, transplantation or death.

2.3 | Transient Elastography and serological non-invasive tests

The TE measurements were made using FibroScan® (Echosens). Valid measurements were those that fulfilled the quality criteria (median value of 10 valid measurements with a ratio >60% and an interquartile range (IQR)/TE < 0.3).[22] Probe M or XL were used according to the patient's body mass index (BMI).[23] Previously published TE cut-offs suggestive of cACLD (TE = 10-15 kPa) or highly suggestive (TE > 15 kPa),[6] and those diagnostic of cirrhosis (TE ≥ 14 kPa)[9,10] were evaluated. Serological NIT as APRI[24] and FIB-4[25] were tested. Reference cut-offs of APRI for exclude/include cirrhosis (<1/>2)[24] and FIB-4 for exclude/include advanced fibrosis (<1.45/>3.25)[25] were assessed.

2.4 | Liver biopsy and histological evaluation

Liver biopsy was performed 3 years after EoT. Biopsy was carried out with abdominal ultrasound guidance using a 16G needle, in accordance with the protocol of each hospital. The samples were formalin-fixed and paraffin-embedded and both haematoxylin-eosin and Masson's Trichrome stains were evaluated.[26] Histological evaluation was centralised and performed by a double-blind reference pathologist (DN) who did not know the patient's clinical/analytical data. Stage of fibrosis was assessed according to the METAVIR score[4] and Laennec grading system.[5] Bridging fibrosis was defined by METAVIR score as F3 and cirrhosis as F4. Advanced fibrosis includes F3 and F4. The Laennec grading system scored patients with cirrhosis as mild (F4A), moderate (F4B) and severe (F4C). Necro-inflammatory activity was assessed according to the METAVIR score and the steatosis percentage followed the NASH CRN system.[4] Serious adverse events (SAEs) associated with liver biopsy defined as any condition causing an increase in hospitalisation (pain, haemoperitoneum, biliary leakage, surgery or death) were registered.

2.5 | Ethical aspects of the study

This study was conducted in accordance with national and international guidelines (deontological code, Declaration of Helsinki 1975) and legal regulations concerning confidential data (LOPD - Spanish Organic Law 15/1999, of 13 December, on the Protection of Personal Data). It was approved by the 'Comité Ético de Investigación Clínica del Parc de Salut Mar' (Parc de Salut Mar Clinical Research Ethics Committee) at the Hospital del Mar with reference number 2017/7492/I, and in the Ethic Committees of the rest of participant hospitals.

2.6 | Sample size and statistical analysis

The sample size was calculated based on D'Ambrosio et al[11] to determine the diagnostic accuracy of TE to identify cirrhosis after SVR (area under the receiver operating characteristics, AUROC = 0.77) with a null hypothesis of AUROC = 0.5, and the publication of Maylin et al[27] to obtain the ratio of negative patients (without cirrhosis) and positive (with cirrhosis) 3 years after SVR (ratio = 9:5). A type I (alpha) error level of 0.05 and a type II (beta) error level of 0.10 were considered, so the number of patients with liver biopsy required was at least 48. It was estimated that up to 90% of patients might refuse to perform the liver biopsy. Therefore, it was planned to include more than 480 patients from April 2018 to April 2019 to demonstrate the diagnostic reliability of TE before and after SVR.

Quantitative variables were expressed as medians (range) and qualitative variables as proportions. The differences between patients with TE suggestive of cACLD (TE 10-15 kPa) vs. those with TE highly suggestive of cACLD (TE > 15 kPa) and patients with suspected cirrhosis (TE ≥ 14 kPa) vs those with TE < 14 kPa before antiviral treatment were analysed with different statistical tests depending on the characteristics of the variables: Fisher's test was used for qualitative variables, and non-parametric tests were used for quantitative variables (the Mann-Whitney *U* test for independent data and the Wilcoxon test for dependent data). Both baseline and 3-year differences among patients who had F4 after SVR and those who had F0-3 were analysed by univariate analysis. All variables with statistical significance ($P < .05$) were included in a multivariate progressive logistic regression analysis to determine independent predictors of post-treatment cirrhosis. Diagnostic

accuracy to identify cirrhosis by TE and NITs (APRI, FIB-4) before and 3 years after antiviral treatment was evaluated with AUROC curves. The AUROC curves were compared using the Hanley-McNeil method.[28]

Sample size and comparisons between AUROC curves were calculated with MedCalc® v19.1.3 (MedCalc Software). All other analyses were performed with SPSS® v20.0 (SPSS Inc).

3 | RESULTS

3.1 | Baseline characteristics of the patients

A total of 746 HCV-infected patients with TE > 9 kPa (95.4% with TE ≥ 10 kPa) before DAA treatment were evaluated. Among these patients, 689 (94.8%) were Child-Pugh A and 38 (5.2%) Child-Pugh B. The median (range) MELD was 6 (6-36). From the total cohort, 670 (89.8%) patients were excluded from performing a liver biopsy 3 years after SVR. Figure 1 shows the detailed flowchart of HCV-infected candidates and the final sample of 76 evaluable subjects. The differences between the patients with (n = 76) and without (n = 670) a liver biopsy 3 years after EoT are depicted in Table 1. Patients who underwent a liver biopsy had a higher percentage of human immunodeficiency virus (HIV) co-infection and more frequent alcohol consumption, with higher median γGT values. However, there were no significant differences between groups in other variables. The proportion of missing values was 1.6% and the mechanism was completely at random. Given the small proportion of missing values, no post hoc method was necessary.[29] Only 2 variables had missing data above 10% (HCV viral load, and alpha-fetoprotein) and were excluded from the analysis.

All patients from the final sample (n = 76) were Child-Pugh A (5) with a median MELD score of 6 (Table 1). No patients presented SAEs resulting from the liver biopsy. Most of the included patients (n = 73, 96.1%) showed a TE > 10 kPa before antiviral treatment and were classified as patients with cACLD: 27 (37%) with TE = 10-15 kPa and 46 (63%) with a TE > 15 kPa.[6]

Table 2 shows the differences between patients with TE suggestive of cACLD (TE = 10-15 kPa) and those highly suggestive of cACLD (TE ≥ 15 kPa). Patients with a TE highly suggestive of cACLD (n = 46) compared to those with only suggestive cACLD (n = 27) had higher bilirubin levels, increased hepatic echogenicity, TE and FIB-4 score values. Moreover, these patients showed lower platelet counts and the proportion of patients who did not show the Baveno VI criteria to avoid endoscopy[6] was higher. There were no significant differences between both groups in other variables. Similar results were found after categorising the patients as suspected cirrhosis with a TE ≥ 14 kPa[9,10] (Table S1).

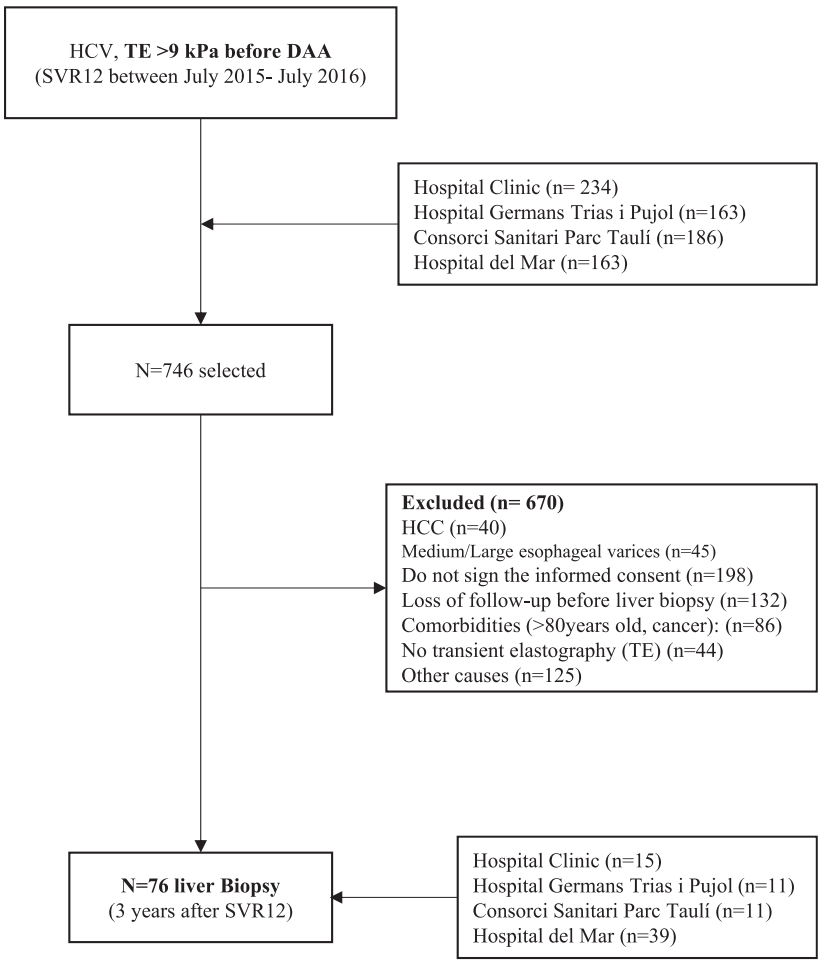


FIGURE 1 Study flowchart

TABLE 1 Baseline characteristics before antiviral treatment (N = 746)

Characteristics N (%)	All patients, N = 746	No liver Biopsy, n = 670 (89.8)	Liver Biopsy, n = 76 (10.2)	*P* value
Males. n (%)	415 (55.7)	367 (54.9)	48 (63.2)	NS
Age. y	63 (18-87)	64 (18-87)	59 (25-80)	NS
BMI, kg/m^2 (n = 656)	26.5 (13.1-46.4)	26.4 (13.1-46.4)	26.6 (16.8-41.4)	NS
Obesity (BMI > 30 kg/m^2), n (%)	161 (24.5)	139 (23.9)	22 (29.3)	NS
T2DM, n (%) (n = 743)	162 (21.8)	145 (21.7)	17 (22.4)	NS
Arterial hypertension, n (%) (n = 715)	206 (28.8)	186 (29.0)	20 (27.0)	NS
Alcohol abuse, n (%) (>20/30 g/dL) (n = 744)	54 (7.3)	48 (7.2)	6 (7.9)	.048
HIV-coinfection, n (%) (n = 743)	30 (4.0)	21 (3.1)	9 (12)	.002
HCV viral load, Log10 IU/mL (n = 737)	6.1 (1.9-7.8)	6.1 (1.9-7.8)	6.1 (3.95-7.38)	NS
HCV genotype, n (%) (n = 737)				NS
1a	114 (15.5)	95 (14.4)	19 (25)	
1b	483 (65.5)	446 (67.5)	37 (48.7)	
2	18 (2.4)	16 (2.4)	2 (2.6)	
3	72 (9.8)	62 (9.4)	10 (13.2)	
4	50 (6.8)	42 (6.4)	8 (10.5)	
TE before treatment, kPa (n = 715)	16.0 (9.1-75.0)	15.7 (9.1-75.0)	19.9 (9.6-43.6)	NS
<10 kPa, n (%)	33 (4.6)	30 (4.7)	3 (3.9)	NS
10-15 kPa, n (%)	302 (42.2)	275 (43)	27 (35.5)	
>15 kPa, n (%)	380 (53.1)	334 (52.3)	46 (60.5)	
Baveno VI criteria, n (%) (n = 742)				NS
No (TE > 20 or PLT < 150)	500 (67.6)	444 (66.7)	56 (73.7)	
Yes (TE < 20 and PLT > 150)	242 (32.6)	222 (33.3)	20 (26.3)	
Abdominal ultrasound, n (%) (n = 733)				
Nodular liver surface	84 (11.5)	71 (10.8)	13 (17.1)	NS
Spleen size >12 cm	305 (41.8)	275 (42.1)	30 (39.5)	NS
Increased hepatic echogenicity	144 (19.6)	130 (19.8)	14 (18.4)	NS
Oesophageal varices, n (%) (n = 384)	194 (50.5)	171 (51.5)	23 (44.2)	NS
AST, IU/L (n = 736)	66.0 (14-670)	66 (14-670)	64.5 (18-333)	NS
ALT, IU/L (n = 740)	71.0 (6-632)	70 (6-632)	76.5 (16-369)	NS
γGT, IU/L (n = 742)	75.5 (13-1867)	73 (13-1867)	98.5 (18-830)	.002
Bilirubin, mg/dL (n = 741)	0.7 (0.2-9.9)	0.7 (0.2-9.9)	0.72 (0.20-6.4)	NS
INR (n = 730)	1.1 (0.9-4.9)	1.1 (0.9-4.9)	1.1 (0.9-1.4)	NS
Albumin, g/dL (n = 740)	4.2 (2.5-5.2)	4.2 (2.5-5.1)	4.2 (3.5-5.2)	NS
Cholesterol, mg/dL (n = 629)	157 (52-292)	157 (52-292)	158 (80-286)	NS
Child-Pugh score (n = 727)				NS
A (5, 6)	689 (94.8)	613 (94.2)	76 (100)	
B (7-9)	38 (5.2)	38 (5.8)	0 (0)	
MELD score (n = 685)	6 (6-36)	6 (6-36)	6 (6-11)	NS
PLT, 10^9/L (n = 743)	138 (10-439)	133 (10-439)	132 (31-386)	NS
<130, n (%)	354 (47.6)	317 (47.5)	37 (48.7)	NS
APRI score (n = 735)	1.5 (0.1-23.3)	1.5 (0.1-23.3)	1.5 (0.28-10.8)	NS
<1, n (%)	236 (32.1)	217 (32.9)	19 (25.0)	NS
1-2, n (%)	236 (32.1)	203 (30.8)	33 (43.4)	

(Continues)

TABLE 1 (Continued)

Characteristics N (%)	All patients, N = 746	No liver Biopsy, n = 670 (89.8)	Liver Biopsy, n = 76 (10.2)	*P* value
>2, n (%)	263 (35.8)	239 (36.3)	24 (31.6)	
FIB-4 score (n = 561)	4.0 (0.3-50.1)	4.0 (0.3-50.1)	4.0 (0.9-18.1)	NS
<1.45, n (%)	44 (7.8)	41 (8.5)	3 (3.9)	NS
1.45-3.25, n (%)	177 (31.6)	149 (30.7)	28 (36.8)	
>3.25, n (%)	340 (60.6)	295 (60.8)	45 (59.2)	
Direct-acting antiviral (DAA)				NS
SOF/LDV	201 (27.5)	180 (28.0)	21 (28.0)	
SOF+DCV	80 (10.9)	69 (10.5)	11 (14.7)	
SOF+SMV	81 (11.1)	70 (10.7)	11 (14.7)	
PTV/r/O/D	333 (45.5)	308 (46.9)	25 (33.3)	
Others	37 (5.1)	30 (4.6)	7 (9.3)	
Ribavirin, n (%)	367 (61.9.0)	320 (61.9)	47 (61.8)	NS
SVR12, n (%)	737 (99.1)	661 (99.0)	76 (100)	NS

Note: Liver biopsies have been collected 3 y after SVR.

Abbreviation: ALT, alanine aminotransferase; APRI, AST to platelet ratio; AST, aspartate aminotransferase; BMI, body mass index; DCV, daclatasvir; FIB-4, fibrosis-4 index; HCV, hepatitis C virus; HIV, human immunodeficiency virus; INR, international normalised ratio; LDV, ledipasvir; MELD, Model for End-Stage Liver Disease; N, number; PLT, platelets; PTV/r/O/D, paritaprevir/ritonavir/ombitasvir/dasabuvir; SMV, simeprevir; SOF, sofosbuvir; SVR, sustained viral response; T2DM, type 2 diabetes mellitus; TE, transient elastography; y, years; γGT, gamma-glutamyl transpeptidase.

3.2 | Impact of SVR on liver function, metabolic-related factors and portal hypertension

Hepatitis C virus elimination was associated with a global improvement in liver function parameters, NITs and TE values 3 years after SVR ($P < .001$ in all cases) (Table 3).

Body mass index evaluation before antiviral treatment was available in 75 (98.7%) patients and 22 of them (29.3%) had obesity (BMI ≥ 30 kg/m^2). Patients with obesity showed a higher proportion of T2DM (40.9% vs. 15.1%; $P = .031$), and higher levels of gamma-glutamyl transpeptidase (γGT) before and after SVR. Among these obese patients, 19 (86.4%) persisted with BMI > 30 kg/m^2, 10 (45.5%) with T2DM and 13 (59.1%) with arterial hypertension after HCV-eradication. After SVR, obese patients showed higher TE values, higher rate of steatosis in ultrasound and higher proportion of patients with >5% of steatosis in liver biopsy. However, obese and non-obese patients showed similar rate of cirrhosis 3 years after SVR. Additional information is depicted in Table S2.

No changes in obesity, type 2 diabetes mellitus (T2DM), or alcohol abuse rates were observed in the whole sample after SVR (Table 4). However, the use of antihypertensive therapy increased (from 20 to 37 patients) after SVR. The Baveno VI criteria to avoid endoscopy were present in 20 patients before antiviral treatment and in 42 after SVR (Table 4). Therefore, oesophageal varices were evaluated in 52 before DAA and in 25 after HCV eradication. Oesophageal varices were present at baseline in 23 (30.3%) but only in 11 (14.5%) after SVR. In patients without oesophageal varices before antiviral treatment, advanced liver fibrosis was present after SVR in 66% of them and 1 (14.3%) out of 7 developed oesophageal varices during follow-up. Supporting Information about patients with or without oesophageal varices is depicted more in detail in Table S3. No patient from our sample (n = 76) developed clinical decompensation or HCC during follow-up.

3.3 | Histological evaluation after SVR

Liver biopsy was performed after 3 years of EoT in the whole sample (median time = 38.4 months). Liver biopsies (median sample size = 24.5 mm) showed absent (A0) or minimal (A1) necro-inflammatory activity in most patients (n = 73, 96%) and non-significant steatosis (<5% of the hepatic parenchyma) in 55 (72.4%) patients. Nevertheless, F4 was observed in 41 (53.9%) and F3 in 12 (15.8%) patients. The fibrosis stage distribution according to Laennec scale is described more in detail in Table 2 and Table S1. Among patients with cirrhosis (n = 41), the Laennec grading system identified F4A in 25 (61%), F4B in 10 (24.4%) and F4C in 6 (14.6%).

3.4 | Characteristics of patients with F4 after SVR

Patients with F4 after SVR (n = 41, 53.9%) showed significant differences compared to those with F0-3 (n = 35, 46.1%; Table 4). Before antiviral treatment, patients with F4 after SVR were younger and had higher rates of HCV-infection by genotype 3, higher bilirubin levels and international normalised ratio (INR) values, lower platelet count, higher APRI and TE values and higher rates of splenomegaly. Three years after HCV elimination, patients with F4 had higher values of

TABLE 2 Characteristics of included patients with cACLD according to TE value before treatment (N = 73)

Characteristics before antiviral treatment, N (%)	All patients, N = 73	TE 10-15 kPa, n = 27 (37)	TE > 15 kPa, n = 46 (63)	*P* value
Males, n (%)	46 (63)	21 (77.8)	25 (54.3)	NS
Age, y	59.2 (25.8-80.4)	57.7 (34.1-78.3)	60.4 (25.8-80.4)	NS
BMI, kg/m^2 (n = 72)	26.5 (16.8-41.4)	26.8 (16.8-39.8)	26.5 (17.9-41.4)	NS
Obesity (BMI > 30 kg/m^2), n (%)	20 (27.8)	8 (29.6)	12 (26.7)	NS
T2DM, n (%)	16 (21.9)	5 (18.5)	11 (23.9)	NS
Arterial hypertension, n (%)	19 (22.5)	8 (29.6)	11 (23.9)	NS
Alcohol abuse (>20/30 g/dL), n (%)	6 (8.2)	1 (3.7)	5 (10.9)	NS
HIV-coinfection, n (%) (n = 72)	9 (12.5)	6 (22.2)	3 (6.7)	NS
HCV genotype 3, n (%)	10 (13.7)	5 (18.5)	5 (10.9)	NS
TE before treatment, kPa	20 (10-43.6)	12 (10-14.3)	21.5 (15.4-43.6)	<.001
Baveno VI criteria, n (%)				<.001
No (TE > 20 or PLT < 150)	55 (75.3)	12 (44.4)	43 (93.5)	
Abdominal ultrasound, n (%)				
Nodular liver surface	13 (18.5)	5 (18.5)	8 (17.4)	NS
Spleen size >12 cm	29 (39.7)	9 (33.3)	20 (43.5)	NS
Increased hepatic echogenicity	14 (19.2)	2 (7.4)	12 (26.1)	.05
Oesophageal varices, n (%) (n = 52)	22 (43.1)	5 (35.7)	17 (45.9)	NS
AST, IU/L	64 (18-333)	56 (18-208)	68.5 (34-333)	NS
ALT, IU/L	78 (16-369)	83 (16-327)	67 (29-369)	NS
γGT, IU/L	98 (20-830)	87 (25-660)	98 (20-830)	NS
Bilirubin, mg/dL	0.7 (0.2-1.7)	0.6 (0.3-1.6)	0.8 (0.2-1.7)	.043
INR	1.1 (0.9-1.4)	1.0 (0.9-1.2)	1.1 (1.0-1.4)	.001
Cholesterol, mg/d (n = 72)	156 (80-236)	156 (115-211)	157 (80-236)	NS
Albumin, g/dL	4.2 (3.5-5.2)	4.3 (3.7-4.9)	4.2 (3.5-5.2)	NS
Child-Pugh score A (5)	73 (100)	27 (100)	46 (100)	NS
MELD score (n = 72)	6 (6-11.4)	6 (6-8.7)	6 (6-11.4)	NS
PLT, 10^9/L	132 (31-386)	156 (31-386)	117 (58-309)	.012
APRI score	1.54 (0.3-10.8)	1.19 (0.3-5.6)	1.67 (0.4-10.8)	NS
<1, n (%)	18 (24.7)	10 (37)	8 (17.4)	
>2, n (%)	23 (31.5)	7 (25.9)	16 (34.8)	
FIB-4 score	3.8 (0.96-18.1)	2.6 (1.0-18.8)	4.3 (1.6-10.2)	.007
<1.45, n (%)	3 (4.1)	3 (11.1)	0 (0.0)	
>3.25, n (%)	43 (58.9)	10 (37)	33 (71.7)	
Liver biopsy 3 y after antiviral treatment				
Obesity (BMI > 30 kg/m^2), n (%)	20 (27.8)	8 (29.6)	12 (26.7)	NS
T2DM, n (%)	17 (23.3)	5 (18.5)	12 (26.1)	NS
Arterial hypertension, n (%)	35 (47.9)	14 (51.9)	21 (45.7)	NS
Alcohol abuse (>20/30 g/dL), n (%)	2 (2.7)	1 (3.7)	1 (2.2)	NS
Time to liver biopsy (months)	38.7 (32-49.1)	38.5 (32.9-46.2)	39.2 (32-49)	NS
Liver biopsy length (mm)	24 (6-40)	32 (12-40)	17 (6-39)	.015
Liver biopsy length ≥15 mm, n (%)	64 (87.7)	25 (92.6)	39 (84.8)	NS
Hepatitis activity (METAVIR), n (%)				NS
No activity (A0)	27 (37)	9 (33.3)	18 (39.1)	
Mild activity (A1)	43 (58.9)	18 (66.7)	25 (54.3)	

(Continues)

TABLE 2 (Continued)

Characteristics before antiviral treatment, N (%)	All patients, N = 73	TE 10-15 kPa, n = 27 (37)	TE > 15 kPa, n = 46 (63)	*P* value
Moderate activity (A2)	2 (2.7)	0 (0.0)	2 (4.3)	
Severe activity (A3)	1 (1.4)	0 (0.0)	1 (2.2)	
Steatosis (NASH CRN), n (%)				NS
<5%	53 (72.6)	21 (77.8)	32 (69.6)	
5%-30%	17 (23.3)	5 (18.5)	12 (26.1)	
30%-60%	3 (4.1)	1 (3.7)	2 (4.3)	
Fibrosis stage (Laeenec), n (%)				.003
F0	2 (2.7)	2 (7.4)	0 (0)	
F1	9 (12.3)	5 (18.5)	4 (8.7)	
F2	11 (15.1)	6 (22.2)	5 (10.9)	
F3	11 (15.1)	6 (22.2)	5 (10.9)	
F4A	24 (32.9)	5 (18.5)	19 (41.3)	
F4B	10 (13.7)	1 (3.7)	9 (19.6)	
F4C	6 (8.2)	2 (7.4)	4 (8.7)	
Cirrhosis (METAVIR, F4), n (%)	40 (54.8)	8 (29.6)	32 (69.6)	.001
Clinical decompensation, n (%)	0 (0)	0 (0)	0 (0)	NS

Abbreviations: ALT, alanine aminotransferase; APRI, AST to platelet ratio; AST, aspartate aminotransferase; BMI, body mass index; FIB-4, fibrosis-4 index; HCV, hepatitis C virus; HIV, human immunodeficiency virus; INR, international normalised ratio; MELD, Model for End-Stage Liver Disease; N, number; NASH CRN, NASH Clinical Research Network; PLT, platelets; T2DM, type 2 diabetes mellitus; TE, transient elastography; y, years; γGT, gamma-glutamyl transpeptidase.

TABLE 3 Impact of HCV eradication on liver function, liver stiffness and NITs in patients with liver biopsy (n = 76)

	Before antiviral treatment	3 y after antiviral treatment	*P* value
AST, IU/L	64.5 (18-333)	21 (9-68)	<.001
ALT, IU/L	76.5 (16-369)	17 (6-70)	<.001
γGT, IU/L	98.5 (18-830)	25.5 (8-157)	<.001
Albumin, g/dL	4.2 (3.5-5.2)	4.6 (3.6-5.2)	<.001
Cholesterol, mg/dL	158 (80-236)	182 (110-239)	<.001
PLT, 10^9/L	132 (31-386)	163 (80-303)	<.001
Bilirubin, mg/dL	0.7 (0.2-1.7)	0.5 (0.1-2.3)	<.001
INR	1.1 (0.9-1.4)	1.1 (1.0-1.3)	NS
TE, kPa	19.9 (9.6-43.6)	10.2 (4.5-37.4)	<.001
APRI score	1.53 (0.3-10.8)	0.4 (0.1-2.1)	<.001
FIB-4 score	3.8 (1.0-18.1)	1.9 (0.6-5.1)	<.001
MELD score	6.0 (6.0-11.4)	6.0 (6.0-11.2)	NS

Abbreviations: ALT, alanine aminotransferase; APRI, AST to platelet ratio; AST, aspartate aminotransferase; FIB-4, fibrosis-4 index; INR, international normalised ratio; MELD, Model for End-Stage Liver Disease; PLT, platelets; TE, transient elastography; γGT, gamma-glutamyl transpeptidase.

aspartate aminotransferase (AST), alanine aminotransferase (ALT), γGT, lower platelet count, higher APRI and TE values and higher rates of splenomegaly than those with F0-3.

Multivariate analysis (OR (95%CI), *P*) showed that HCV-infection by genotype 3 (20.81 (2.12-201.47), .009), and TE (kPa) before antiviral treatment (1.21 (1.09-1.34), <.001) were the only variables independently associated with cirrhosis 3 years after SVR. Characteristics of HCV-genotype 3 infected patients are reported in Table S4.

3.5 | Diagnostic accuracy of non-invasive methods to identify F4 after SVR

Patients with a TE ≥ 15 kPa before antiviral treatment showed higher proportion of F4 after SVR compared to those with a TE = 10-15 kPa (69.6% vs 29.6%; *P* = .001) (Table 2). None of the patients (0%) with TE ≥ 15 kPa before DAA completely eliminated liver fibrosis (F0) 3 years after HCV eradication. The absence of fibrosis was observed in two patients (7.4%) with TE = 10-15 kPa (*P* = .003). Similar results were found in patients with suspected cirrhosis before antiviral treatment (TE ≥ 14 kPa) compared to those with a TE < 14 kPa (Table S1). However, one out of three patients with a TE 9.6-9.9 kPa before antiviral treatment showed F4 after HCV eradication.

Diagnostic accuracy of TE, APRI and FIB-4 before and 3 years after SVR to identify patients with F4 was evaluated with the AUROC curve and the comparative analysis with the Hanley-McNeil method[28] (Figure 2). The best variable for predicting F4 after SVR was TE before the antiviral treatment (AUROC = 0.79), being the accuracy lower for APRI (AUROC = 0.71) and FIB-4 (AUROC = 0.64)

TABLE 4 Characteristics of included patients (N = 76) and variables associated with METAVIR F4, 3 y after antiviral treatment

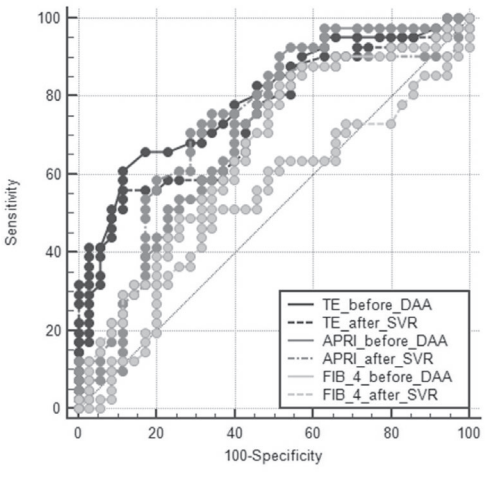
Characteristics before antiviral treatment	METAVIR F0-3 (n = 35), 46.1%	METAVIR F4 (n = 41), 53.9%	*P* value	OR (95% CI), *P*
Males, n (%)	23 (65.7)	25 (61.0)	NS	
Age, y	68.0 (34.3-80.7)	54.9 (26.3-77.6)	<.001	
<65, n (%)	15 (42.9)	33 (80.5)	.001	
Obesity (BMI > 30 kg/m^2), n (%) (n = 75)	8 (22.9)	14 (35)	NS	
T2DM, n (%)	6 (17.1)	11 (26.8)	NS	
Arterial hypertension, n (%)	10 (28.6)	10 (24.4)	NS	
Alcohol abuse (>20/30 g/dL), n (%)	1 (2.9)	5 (12.2)	NS	
Child-Pugh score A (5)	35 (100)	41 (100)	NS	
MELD score (n = 75)	6 (6-11.4)	6 (6-8.7)	NS	
HIV-coinfection, n (%)	4 (11.4)	5 (12.5)	NS	
HCV Genotype 3, n (%)	1 (2.9)	9 (22.0)	.017	20.8 (2.12-201), .009
AST, IU/L	60 (18-208)	72 (34-333)	NS	
ALT, IU/L	70 (16-327)	82 (29-369)	NS	
γGT, IU/L	87 (18-660)	105 (43-830)	NS	
Bilirubin, mg/dL	0.6 (0.3-1.62)	0.9 (0.2-1.7)	.023	
INR	1.06 (0.9-1.2)	1.1 (1-1.4)	.022	
Albumin, g/dL	4.2 (3.7-4.9)	4.2 (3.5-5.2)	NS	
Abdominal ultrasound, n (%)				
Nodular liver surface	7 (20)	6 (14.6)	NS	
Spleen size >12 cm	8 (22.9)	22 (53.7)	.006	
Increased hepatic echogenicity	6 (17.1)	8 (19.5)	NS	
Oesophageal varices, n (%) (n = 52)	6 (28.6)	17 (54.8)	.061	
PLT, 10^9/L	160 (31-386)	115 (58-253)	.001	
<160, n (%)	16 (45.7)	34 (82.9)	.001	
APRI score	1.1 (0.28-6.2)	1.8 (0.49-10.8)	.002	
<1, n (%)	16 (45.7)	3 (7.3)	.001	
>2, n (%)	7 (20.0)	17 (41.5)		
FIB-4 score	3.4 (1.1-18.8)	4.6 (1.0-11.0)	NS	
<1.45, n (%)	2 (5.7)	1 (2.4)	NS	
>3.25, n (%)	16 (45.7)	29 (70.7)		
TE before treatment, kPa	14 (9.8-30.8)	21.3 (9.6-43.6)	<.001	1.21 (1.09-1.34), <.001
<10 kPa, n (%)	2 (5.7)	1 (2.4)	.002	
10-15 kPa, n (%)	19 (54.3)	8 (19.5)		
>15 kPa, n (%)	14 (40)	32 (78)		
Baveno VI criteria, n (%)			.002	
No (TE > 20 or PLT < 150)	20 (57.1)	36 (87.8)		
Characteristics 3 y after antiviral treatment				
Obesity (BMI > 30 kg/m^2), n (%)	9 (25.7)	13 (32.5)	NS	
T2DM, n (%)	6 (17.1)	12 (29.3)	NS	
Arterial hypertension, n (%)	19 (54.3)	18 (43.9)	NS	
Alcohol abuse (>20/30 g/dL), n (%)	1 (2.9)	1 (2.4)	NS	
AST, IU/L	20 (12-37)	24 (9-68)	.01	
ALT, IU/L	15 (6-56)	21 (11-70)	.001	

(Continues)

TABLE 4 (Continued)

Characteristics before antiviral treatment	METAVIR F0-3 (n = 35), 46.1%	METAVIR F4 (n = 41), 53.9%	*P* value	OR (95% CI), *P*
γGT, IU/L	22 (8-90)	37 (12-157)	.006	
Bilirubin, mg/dL (n = 75)	0.5 (0.3-1.5)	0.6 (0.1-2.3)	NS	
INR (n = 75)	1.08 (1-1.2)	1.05 (0.9-1.3)	NS	
Albumin, g/dL (n = 74)	4.5 (3.6-5.2)	4.6 (3.9-5.2)	NS	
PLT, 10^9/L	190 (97-303)	143 (80-296)	.004	
Abdominal ultrasound, n (%) (n = 74)				
Nodular liver surface	1 (2.9)	3 (7.5)	NS	
Spleen size >12 cm	4 (11.8)	13 (32.5)	.035	
Increased hepatic echogenicity	4 (11.8)	11 (27.5)	.093	
Oesophageal varices, n (%) (n = 25)	1 (9.1)	10 (50.0)	NS	
APRI score	0.2 (0.1-0.7)	0.4 (0.1-2.1)	NS	
<1, n (%)	35 (100)	39 (95.1)	NS	
>2, n (%)	0 (0.0)	1 (2.4)		
FIB-4 score	2.0 (0.8-3.4)	2.5 (0.5-5.5)	NS	
<1.45, n (%)	12 (34.3)	14 (34.1)	NS	
>3.25, n (%)	2 (5.7)	6 (14.6)		
TE before liver biopsy, kPa	8.6 (4.5-15.2)	13.2 (5.4-37.4)	<.001	
<8 kPa, n (%)	15 (45.5)	6 (14.6)	.003	
>8 kPa, n (%)	18 (54.5)	35 (85.4)		
Baveno VI criteria, n (%) (n = 74)			.004	
No (TE > 20 or PLT < 150)	9 (25.7)	25 (61.0)		

Abbreviations: ALT, alanine aminotransferase; APRI, AST to platelet ratio; AST, aspartate aminotransferase; FIB-4, fibrosis-4 index; HCV, hepatitis C virus; HIV, human immunodeficiency virus; INR, international normalised ratio; N, number; PLT, platelets; T2DM, type-2 diabetes mellitus; TE, transient elastography; y, years; γGT, gamma-glutamyl transpeptidase.



[a] Hanley & McNeil, 1982

Variable	AUROC	95% CI
TE before DAA	**0.786**[*,**]	**0.677 - 0.872**
TE after SVR	0.747	0.634 – 0.840
APRI before DAA	0.706	0.590 – 0.805
APRI after SVR	0.715	0.600 – 0.813
FIB-4 before DAA	0.638[*]	0.520 – 0.745
FIB-4 after SVR	0.553[**]	0.434 – 0.667

*,**The differences between AUROCs are statistically significant

FIGURE 2 AUROC curves of TE accuracy for the diagnosis of cirrhosis in patients with liver biopsy. The corresponding areas under the AUROC curves were 0.79 (95% CI: 0.68-0.87) for TE before DAA, 0.75 (95% CI: 0.63-0.84) for TE after SVR, 0.71 (95% CI: 0.59-0.81) for APRI before DAA, 0.71 (95% CI: 0.60-0.81) for APRI after SVR, 0.64 (95% CI: 0.52-0.75) for FIB-4 before DAA, and 0.55 (95% CI: 0.43-0.67) for FIB-4 after SVR. The AUROC curves were compared using the Hanley-McNeil method[28] and the differences statistically significant are depicted

before treatment, and TE (AUROC = 0.75), APRI (AUROC = 0.71) and FIB-4 (AUROC = 0.55) 3 years after SVR. The best cut-off of TE before antiviral treatment to identify patients with F4 after SVR was 20.9 kPa (sensitivity 61% and specificity 88.6%), but the cut-off with the highest NPV was 12 kPa (NPV = 88%; Figure 3). Among 19 patients with APRI <1 before treatment, 3 (15.8%) revealed F4 and 5 (26.3%) F3, meaning that up to 8 (42%) patients with an APRI <1 and TE > 10 kPa before treatment, showed F3-4, 3 years after SVR. Among 24 patients with APRI > 2 before antiviral therapy, 17 (70.8%) had F4 and 4 (16.7%) F3 thereafter. Out of 33 patients with intermediate APRI, 21 (63.6%) had F4 and 3 (9.1%) had F3. Similar results were found with FIB-4 test (Figure S1).

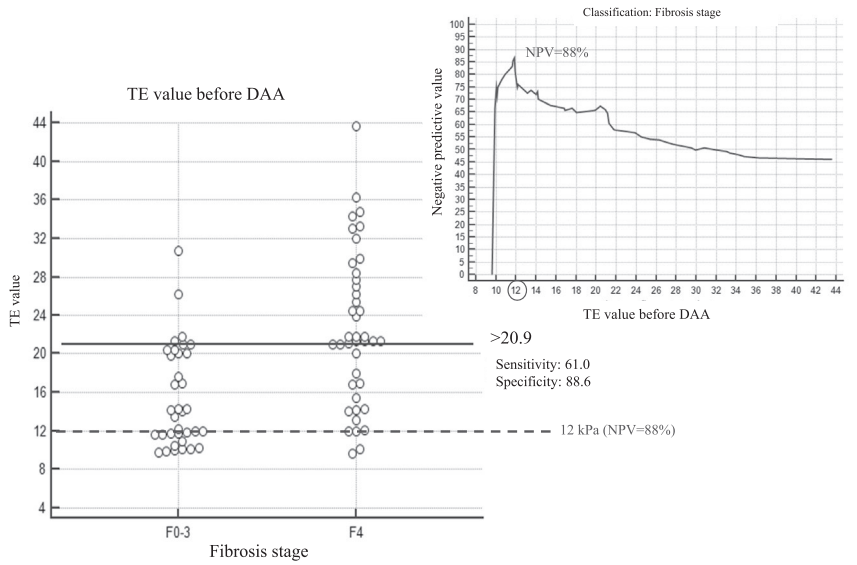


FIGURE 3 Best cut-off points for TE (kPa) before DAA treatment. The best cut-off of TE before antiviral treatment to identify patients with cirrhosis after SVR was 20.9 kPa (sensitivity 61% and specificity 88.6%), but the cut-off with the highest negative predictive value (NPV) was 12 kPa (NPV = 88%)

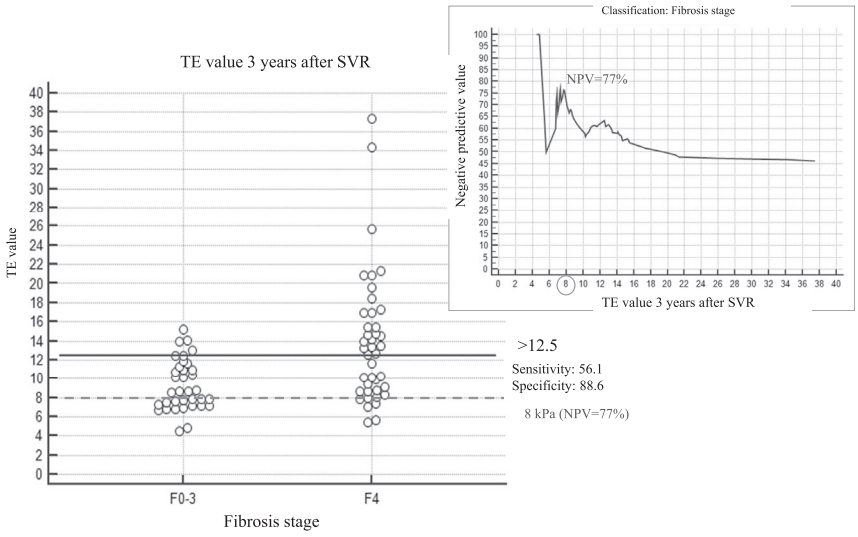


FIGURE 4 Best cut-off points for TE (kPa) 3 y after SVR. The best cut-off of TE 3 y after SVR to identify cirrhosis was 12.5 kPa (sensitivity 56.1% and specificity 88.6%), but the value with the highest NPV was 8 kPa (NPV = 77%)

The elimination of HCV after antiviral treatment produced a significant decrease in TE, APRI and FIB-4 values. The best cut-off for the TE 3 years after SVR to identify F4 was 12.5 kPa (sensitivity 56.1% and specificity 88.6%), but the value with the highest NPV was 8 kPa (NPV = 77%; Figure 4). In consequence, 12 (37.5%) out of 32 patients with a TE 3 years after EoT <9.5 kPa showed F4 and 19 (59.4%) F3-4. Among 22 patients with a TE value <8 kPa 3 years after SVR, 12 (54.5%) revealed F3-4: 6 (27.3%) F3 and 6 (27.3%) F4 (Figure S2). Similarly, NITs experienced a significant decrease after HCV elimination. We did not find differences in FIB-4 or APRI values after SVR between patients with F4 and those F0-3.

4 | DISCUSSION

Our work is probably the largest prospective multi-centric study of HCV-cACLD patients with TE paired measurements before treatment and 3 years after SVR with DAA and liver biopsy. We have shown that neither TE nor NITs as APRI or FIB-4 are reliable enough to accurately identify liver fibrosis stage after SVR. In spite of finding significant improvements in liver function parameters and TE values after HCV clearance, cirrhosis was present in more than half of the patients and advanced fibrosis in 70%.

Our study assessed a large multicentre cohort of HCV-infected cACLD patients before antiviral treatment. Total cohort showed indirect signs of portal hypertension in more than half of them and one-third were obese patients with T2DM. Patients with suspected cirrhosis or with a TE value highly suggestive of cACLD before antiviral treatment[6] showed the highest proportion of F4, 3 years after SVR. Obese patients showed higher values of TE, increased hepatic echogenicity and steatosis despite HCV eradication, but no differences in liver fibrosis stage were found. Unfortunately, the acceptance to undergo the liver biopsy after HCV elimination was low in real clinical practice. Nevertheless, the multicentre origin of the

samples and the comparative baseline analysis demonstrated that patients who underwent the liver biopsy were representative of the total cohort.

Our work has demonstrated that HCV genotype 3 and high TE values before treatment were independently associated with cirrhosis 3 years after SVR. Patients infected by genotype 3 were younger and had a higher percentage of HIV-coinfection. The HCV-genotype 3 and HIV coinfection have been associated with a faster liver fibrosis progression, higher degree of portal hypertension and an increased incidence of HCC.[30-33] However, prospective studies evaluating fibrosis regression after SVR in HIV and HCV coinfected patients are scarce, especially after DAA introduction. Labarga et al demonstrated progression in 7.2% of HIV/HCV patients despite SVR with pegylated interferon/ribavirin (median time: 4.4 years).[34] However, this study was retrospective and only 32% had advanced fibrosis. Our prospective study has demonstrated the presence of advanced fibrosis 3 years after SVR in most HIV co-infected patients and in all previously infected by GT3.

The best TE cut-off point before antiviral treatment to differentiate our patients with cirrhosis after SVR was 20.9 kPa (sensitivity 61% and specificity 88.6%). However, the cut-off point with the highest negative predictive value was 12 kPa (NPV = 88%) and 1 out of 3 patients with a TE 9.6-9.9 kPa showed F4 after SVR. The elimination of the HCV infection produced a significant decrease in ALT, AST, FIB-4, APRI and TE values 3 years after antiviral treatment. However, liver biopsies showed cirrhosis in 54% of them. A systematic review including 24 studies with paired TE before and after SVR has shown that liver stiffness decreases around 3 kPa in the first 6-12 months following HCV clearance.[35] Our study showed that 47% of patients with a baseline TE compatible with advanced fibrosis (TE > 9.5 kPa) presented values <9.5 kPa after SVR.[35] Likewise, the best cut-off point to differentiate patients with liver cirrhosis decreased to 12.5 kPa 3 years after SVR (sensitivity 56.1% and specificity 88.6%) and the lowest cut-off of 8 kPa only showed a moderate NPV (77%). Although this early decrease in TE is related to improvements in inflammation and necrosis, there are few evidences that suggest an improvement in the stage of liver fibrosis.[7] A recent pilot study evaluating 15 paired biopsies by morphometry confirms fibrosis regression after SVR, but showed important discordances with the decrease in TE values: 13 out of 15 patients with high TE values before treatment, showed a TE <9.5 kPa after SVR, but only 4 had low liver fibrosis stages (<F3-F4).[15] Despite this inconclusive data, international guidelines recommend the use of TE to follow up patients after SVR[7,21] and The American Gastroenterological Association hypothesise that a post-treatment TE cut-off of 9.5 kPa can rule out advanced liver fibrosis after SVR allowing these patients to be discharged.[7] However, our results indicate that TE after SVR is not reliable enough to correctly classify patients with cirrhosis. Similarly, NITs decreased after SVR; however, their correlation with the histological fibrosis stage was low. In this line, a recent study has shown that patients with cirrhosis before antiviral treatment have a high risk of developing HCC after SVR, even among those in whom their FIB-4 score decreases, and authors recommend the indefinite HCC surveillance.[36]

Recent studies have shown that the risk of persistent portal hypertension and developing liver-related complications still exist after SVR in patients with HCV- cACLD. Lens et al[16] showed the persistence of clinically significant portal hypertension (CSPH) at SVR24 in up to 80% of patients with CSPH before DAA, but TE values were not sufficiently discriminatory to rule out CSPH. Similarly, our group[18] has demonstrated that HCV-cirrhotic patients can develop high-risk gastroesophageal varices after SVR, especially in those with high TE values or gastroesophageal varices before treatment. In consonance, the current study has demonstrated that 1 out of 7 HCV-cACLD patients without oesophageal varices can develop 'the novo' varices despite HCV elimination. However, none of our patients developed clinical decompensation or HCC, although this could be associated with the restrictive exclusion criteria for the liver biopsy. Recently, Pons et al[37] have shown that HCC is the most frequent liver-related complication after SVR, and authors recommend indefinite surveillance for HCC in patients with HCV- cACLD before treatment. In this line, our study has demonstrated that 70% of HCV-cACLD patients have F3-F4 after 3 years of viral eradication, and therefore, ultrasound follow-up is recommended.

The limitations of our study were (a) the lack of biopsies before antiviral therapy that prevented us to analyse the exact rate of fibrosis regression/progression after SVR; however, this fact was expected since current international guidelines[9,10] accept TE as the best non-invasive method to identify patients with cirrhosis before antiviral therapy; (b) the low patients' acceptance rate for liver biopsies in clinical practice after HCV eradication, which implied a reduced sample size and (c) the lack of external validation, which do not allow to give solid recommendations regarding the optimal follow-up of these patients. Indeed, we can only suggest upper endoscopy for variceal surveillance in the group of patients at higher risk of cirrhosis after SVR, but this would clearly need external validation in a larger sample.

In spite of this, we believe that our study has important strengths. This is probably the largest prospective multi-centric study of HCV-cACLD patients evaluated 3 years after DAA with simultaneous liver biopsy, TE and non-invasive tests. Moreover, the multicentre origin of the histological samples, the good quality and the centralised evaluation, made biopsied patients as representative of the total cohort.

In conclusion, our study has shown that despite significant improvements in liver function parameters, NITs and TE values can be observed after HCV clearance in HCV-cACLD patients, cirrhosis and advanced fibrosis is present in 50% and 70%, respectively, after 3 years of viral elimination. Neither TE values nor NITs, as APRI or FIB-4, are reliable enough to accurately classify the liver fibrosis stage after SVR. The low diagnostic accuracy of non-invasive methods and the persistent risk of developing liver-related complications after SVR prompt us to recommend continuous surveillance in those patients with advanced chronic liver disease before treatment, being still the liver biopsy the only reliable method to exclude advanced fibrosis or cirrhosis after SVR.

CONFLICT OF INTEREST

TB: no conflict of interest; PH: no conflict of interest; ZM: Speaker fees and consultancy for Gilead and AbbVie; DN: no conflict of interest; MV: Speaker fees from AbbVie, Gilead and Intercept; RMM: Speaker fees from Gilead, AbbVie, Intercept and Advisory boards for Intercept; XF: Speaker fees from AbbVie and Gilead; JAC: Speaker fees from AbbVie and Gilead.

AUTHOR CONTRIBUTION

TB and PH: data collection, analysis and interpretation of data; statistical analysis; drafting and critical revision of the manuscript. ZM, MV, RMM: data collection and critical revision of the manuscript. DN: histological evaluation of liver biopsies; data collection and critical revision of the manuscript. XF: study concept and design; data collection and critical revision of the manuscript. JAC: study concept and design; data collection; statistical analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript; and study supervision.

DATA AVAILABILITY STATEMENT

No additional data are available.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Broquetas T, Herruzo-Pino P, Mariño Z, et al. Elastography is unable to exclude cirrhosis after sustained virological response in HCV-infected patients with advanced chronic liver disease. *Liver Int*. 2021;41:2733–2746. https://doi.org/10.1111/liv.15058