Alanine aminotransferase and spleno-portal dynamics affect spleen stiffness measured by point shear-wave elastography in patients with chronic hepatitis C in the absence of significant liver fibrosis

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Accepted: 27 March 2020

Abstract

Background Spleen stiffness (SS) has gained a lot of interest in the context of liver cirrhosis and portal hypertension stratification. However, there is a paucity of data on confounding factors that may alter SS values.

Methods Between January 2018 and October 2019, we enrolled 120 healthy subjects and 117 patients with hepatitis C virus (HCV) infection who did not have significant liver fibrosis (i.e., F0–1). Abdominal ultrasound evaluation was performed on each individual to measure portal vein diameter, portal flow velocity, spleen bipolar diameter, and splenic area. We also performed liver and spleen elastography.

Results HCV patients had higher SS ($p < 0.001$), portal vein diameter ($p = 0.031$), portal flow velocity ($p = 0.035$), spleen bipolar diameter ($p = 0.042$) and area ($p = 0.025$), and ALT levels ($p < 0.001$). Linear regression models showed that SS increased by 3.220 kPa for each mm of portal vein diameter, by 0.7 kPa for each cm/s of portal flow velocity, by 2.239 kPa for each cm of spleen bipolar diameter, and by 0.233 kPa for each cm^2 of spleen area. Patients with HCV infection were stratified according to median ALT levels (i.e. 32 IU/L). SS and spleno-portal axis parameters were significantly higher in patients with an ALT level > 32 IU/L. Besides, the relationship between SS and ALT was described by cubic polynomial regression according to the following equation: $11.735 + 0.404\ (ALT)^1 - 0.002\ (ALT)^2 + 4.26 \times 10^{-6}\ (ALT)^3$.

Conclusions Our results bring new light to the role of inflammation as a confounding factor for SS measurement. Therefore, particular attention should be paid to serum transaminase for a correct evaluation of spleen elastography.

Keywords Spleen stiffness · Liver stiffness · Serum transaminase · ALT · Portal vein · Spleen

Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease, with a natural history that is highly variable ranging from minimal histological changes to liver cirrhosis and its complications [1]. Clinical care for patients with HCV infection has advanced considerably during the last 2 decades, in light of improvements in diagnostic and staging procedures and, above all, by the introduction of direct-acting antiviral (DAA) therapy. However, proper assessment of liver disease severity (i.e., quantification of liver fibrosis) is necessary before therapy [2, 3]. Over the past decade, liver elastography has made its way through non-invasive techniques (NITs), becoming the best surrogates for staging liver fibrosis [4]. Liver stiffness (LS) is now well validated and ready to be safely used in everyday clinical practice, especially in the first-line fibrosis staging in patients infected by HCV [2, 5]. However, guidelines suggested that non-invasive tests should always be interpreted by hepatologists in light of the clinical context and other biochemical, radiological, and endoscopic tests. In fact, when interpreting elastography results, one should be aware that stiffness values do not only depend on fibrosis or

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parenchymal congestion but acute inflammation as well [6, 7]; hence, the need, albeit controversial, to adjust LS values to transaminase levels (especially in patients with an apparently higher degree of fibrosis [8]) or to complement LS measurements with other NITs [9, 10].

Spleen elastography has come to the aid of LS, in particular in the context of portal hypertension and non-invasive screening of esophageal varices [11]. Healthy values of spleen stiffness (SS) have been evaluated by a few authors using different elastography techniques [12]. Besides, SS has also been associated with different stages of liver fibrosis [13]. SS seems to be affected by two principal factors: parenchymal congestion and fibrosis. In particular, the congestion-induced increment of SS has been proved both directly, by its close relationship with portal pressure measurements [14], and indirectly, by the reduction of SS due to TIPS placement [15] or non-selective β-blocker (NSBB) therapy [16]. Also, Ravaioli et al. [17] reported a significant decrease in SS after successful antiviral treatment suggesting that SS may have been affected by acute inflammation, or conversely remained unaltered and was able to predict esophageal varices [18].

That being said, the aim of this study was to evaluate if HCV-induced increase in serum transaminase and changes in spleno-portal dynamics can affect SS measurement. Therefore, we compared SS between patients affected by HCV who did not have significant liver fibrosis and a group of healthy controls.

Materials and methods

The study was carried out retrospectively following the guidelines and formal approval of the local Ethics Committee for conducting research involving humans (ID: 2783, Comitato Etico Unico Regionale, Friuli Venezia Giulia). The study was conducted according to the criteria set by the Declaration of Helsinki and each subject signed an informed consent form before participating in the study. Patients and healthy subjects were referred to the Liver Clinic between January 2018 and October 2019. We have already explained elsewhere how the ultrasound and elastography examinations were performed with the Philips Affiniti 70 instrument [11]. We defined unreliable examinations as those with interquartile range/median ratio > 0.30 [4]. Ultrasonography and elastography were conducted after a mandatory period of at least three hours of fasting [19, 20].

Healthy subject selection criteria

Individuals consisted mostly of hospital staff, students, or their relatives older than 18 years. We also included data from a cohort of 100 healthy individuals mentioned in a previous study [12]. They were all volunteers who had no substantial past medical history of acute or chronic liver disease and/or hematological disorder. However, to avoid possible confounding factors, we included individuals with normal values of serum transaminase (< 30 IU/L), gamma-glutamyl transpeptidase (< 40 IU/L), and alkaline phosphatase (< 150 IU/L). In addition, they must have had no substantial alcohol intake (< 30 g/daily for men, < 20 g/daily women) and negative hepatitis B, hepatitis C, and HIV blood serology. This original group underwent a full ultrasonographic and Doppler screening for the search of splenomegaly, focal splenic/hepatic lesions, degree of steatosis, and signs of portal hypertension. In particular, we excluded patients with (1) spleen bipolar diameter > 12 cm (females) or > 13 cm (males); (2) focal splenic and hepatic lesions; (3) moderate/severe liver steatosis (Hamaguchi score ≥ 3 [21]); (4) presence of liver coarse echotexture, surface nodularity or caudate lobe hypertrophy [22]); (5) one of the following: loss of triphasic waveform in hepatic veins, hepato-fugal flow in portal vein, portal vein/mesenteric vein/splenic vein dilation, or signs of hepatofugal shunts [23]; (6) documented congestive hepatopathy. Finally, patients with LS values > 6.34 kPa [24] were excluded from SS measurement.

HCV patient selection criteria

The criteria included were (1) age > 18 years, (2) HCV infection confirmed by positive HCV-RNA titers, (3) intention to treat with DAA, but uninitiated treatment, and (4) absence of significant liver fibrosis ($F = 0$–1, LS < 6.34 kPa) [24]. We categorically excluded patients with current HBV and/or HIV co-infection, a history of autoimmune hepatitis, non-alcoholic fatty liver disease (NAFLD), hemochromatosis, or Wilson's disease and other liver storage disorders. In addition, other exclusion criteria regarded the risk of having false negatives for F0–1 stages (i.e., some patients may show lower LS values when compared to the progression of their liver disease), and the study of factors that could influence spleno-portal dynamics. Therefore, we excluded pregnant women, patients with current alcohol abuse, patients with current or past decompensating events (such as hepatic encephalopathy, variceal hemorrhage, ascites, and spontaneous bacterial peritonitis), previous endoscopic EV banding ligation, an ongoing intake of non-selective beta-blockers (NSBB), a history of portal vein thrombosis, placement of transjugular intrahepatic portosystemic shunt (TIPS), non-cirrhotic causes of PH, a current/recent diagnosis of hepatocellular carcinoma [25], presence of collateral hepatofugal shuntings, and signs of liver failure. We also excluded patients with heart failure and/or documented congestive hepatopathy.

Statistical analysis

Because of the size of our sample, the Shapiro–Wilk test was performed to verify the normal distribution of variables. Accordingly, normally distributed variables were reported as mean (± standard deviation, SD), whereas other variables were reported as median (Quartile 1;Quartile 3). Differences between continuous variables (healthy subjects vs. HCV patients, in addition to HCV patients stratified by median ALT values) were examined using the Student's *t* test (if normally distributed) or Mann–Whitney *U* test (if not normally distributed).

The correlation between SS and spleno-portal axis parameters was assessed using the Spearman rank-order correlation coefficient [26]. Linear regression analysis was performed to describe the linear relationship between SS (dependent variable) and spleno-portal axis (independent variables) [27]. Collinearity for a multiple linear regression model was assessed in terms of the variance inflation factor (VIF) [28]. HCV-positive patients were stratified according to median values for ALT (i.e., 32 U/L) to determine statistically significant differences between patients with elevated serum ALT vs. normal serum ALT. Cubic polynomial regression analysis was performed to assess the model between SS (dependent variable) and ALT levels (independent variable). For all analyses, two-sided statistical significance was defined as $p < 0.05$. Data were analyzed using SPSS (Statistical Package for Social Science) version 25.0 (IBM SPSS Statistics for MAC OS. Armonk, NY: IBM Corp.).

Results

We initially enrolled 140 healthy subjects, of which eight had LS values > 6.34 kPa and 12 had unreliable SS measures. As a result, 120 patients were selected, which consisted of 59 (49.2%) females with a median age of 53 (28;56) years. The median LS was 5 (4.11;5.62) kPa and the median SS was 17.63 (15.8;21) kPa. The evaluation of the spleno-portal axis resulted in a median portal vein diameter of 10.3 (9.1;11.3) mm, a median portal flow velocity of 19.9 (17.6;21.5) cm/s, a median spleen bipolar diameter of 10.3 (9.31;11.2) cm, and a median spleen area of 38.1 (32;42.5) cm^2. The medial ALT levels were 20 (12;30) IU/L.

Regarding HCV patients, from an original cohort of 301 individuals, only 130 had LS values < 6.34 kPa, of which 13 had unreliable SS measures. Therefore, 117 patients were selected, which mostly consisted of female individuals ($n = 62$, 53%) with a median age of 62 (55;75.5) years. The median LS was 5.2 (4.20;5.81) kPa, and the median SS was 23.6 (19.2;26.5) kPa. Ultrasonographic examination of the spleno-portal axis showed a median portal vein diameter of 11.1 (9.1;12.1) mm, a median portal flow velocity of 22.5 (18.5;26.5) cm/s, a median spleen bipolar diameter of 11.5 (10.5;12.95) cm, and a medial spleen area of 45 (31.7;54) cm^2. The median ALT levels were 32 (19;59) IU/L.

As reported in Table 1, statistically significant differences were registered between the two groups of healthy subjects and HCV patients in terms of age ($p < 0.001$), SS ($p < 0.001$), portal vein diameter ($p = 0.001$), portal flow velocity ($p = 0.035$), spleen bipolar diameter ($p = 0.014$), and serum ALT ($p < 0.001$).

Spleen stiffness and the spleno-portal axis

Based on the results of the study, SS in healthy subjects tends to be higher in individuals with increased portal flow velocity ($r_s = 0.457$, $p = 0.05$) and lower in patients with increased splenic area ($r_s = -0.215$, $p = 0.03$). Besides, SS of patients affected by HCV tends to be higher in individuals with increased portal vein diameter ($r_s = 0.689$, $p < 0.001$), portal flow velocity ($r_s = 0.671$, $p < 0.001$), spleen bipolar diameter ($r_s = 0.736$, $p < 0.001$), and spleen

Table 1 Differences between healthy subjects and patients with HCV infection

Variables	Healthy subjects $n = 120$	HCV patients $n = 117$	Significance
Sex, female	59 (49.2%)	62 (53%)	NS
Age (years)	53 (28; 56)	62 (55;75.5)	$p < 0.001$
Liver stiffness (kPa)	5 (4.11;5.62)	5.2 (4.20;5.81)	NS
Spleen stiffness (kPa)	17.63 (15.8;21)	23.6 (19.2;28)	$p < 0.001$
Portal vein diameter (mm)	10.3 (9.1;11.3)	11.1 (9.1;12.1)	$p = 0.031$
Portal flow velocity (cm/s)	19.9 (17.6;21.5)	22.5 (18.5;26.5)	$p = 0.035$
Spleen bipolar diameter (cm)	10.3 (9.31;11.2)	11.5 (10.5;12.95)	$p = 0.042$
Spleen area (cm^2)	38.1 (32; 42.5)	45 (31.7;54)	$p = 0.025$
ALT (IU/L)	20 (12;30)	32 (19;59)	$p < 0.001$

According to statistical analyses, HCV patients showed higher spleen stiffness, portal vein diameter, portal flow velocity, spleen bipolar diameter, spleen area, and ALT

area ($r_s = 0.757$, $p < 0.001$). Simple linear regressions were calculated to predict SS based on spleno-portal axis parameters (Fig. 1). Regarding portal vein diameter, a significant regression equation was found ($F(1,115) = 93.348$, $p < 0.001$), with an R^2 of 0.448. Participants' predicted SS is equal to [− 9.264 + 3.220 (portal vein diameter)] kPa when portal flow velocity is measured in mm. SS increased 3.220 for each mm of portal vein diameter. Regarding portal flow velocity, a significant regression equation was found ($F(1,115) = 88.248$, $p < 0.001$), with an R^2 of 0.434. Patients' predicted SS is equal to [7.888 + 0.7 (portal flow velocity)] kPa when portal flow is measured in cm/s. SS increased 0.7 for each cm/s of portal flow velocity. In relation to spleen bipolar diameter, a significant regression equation

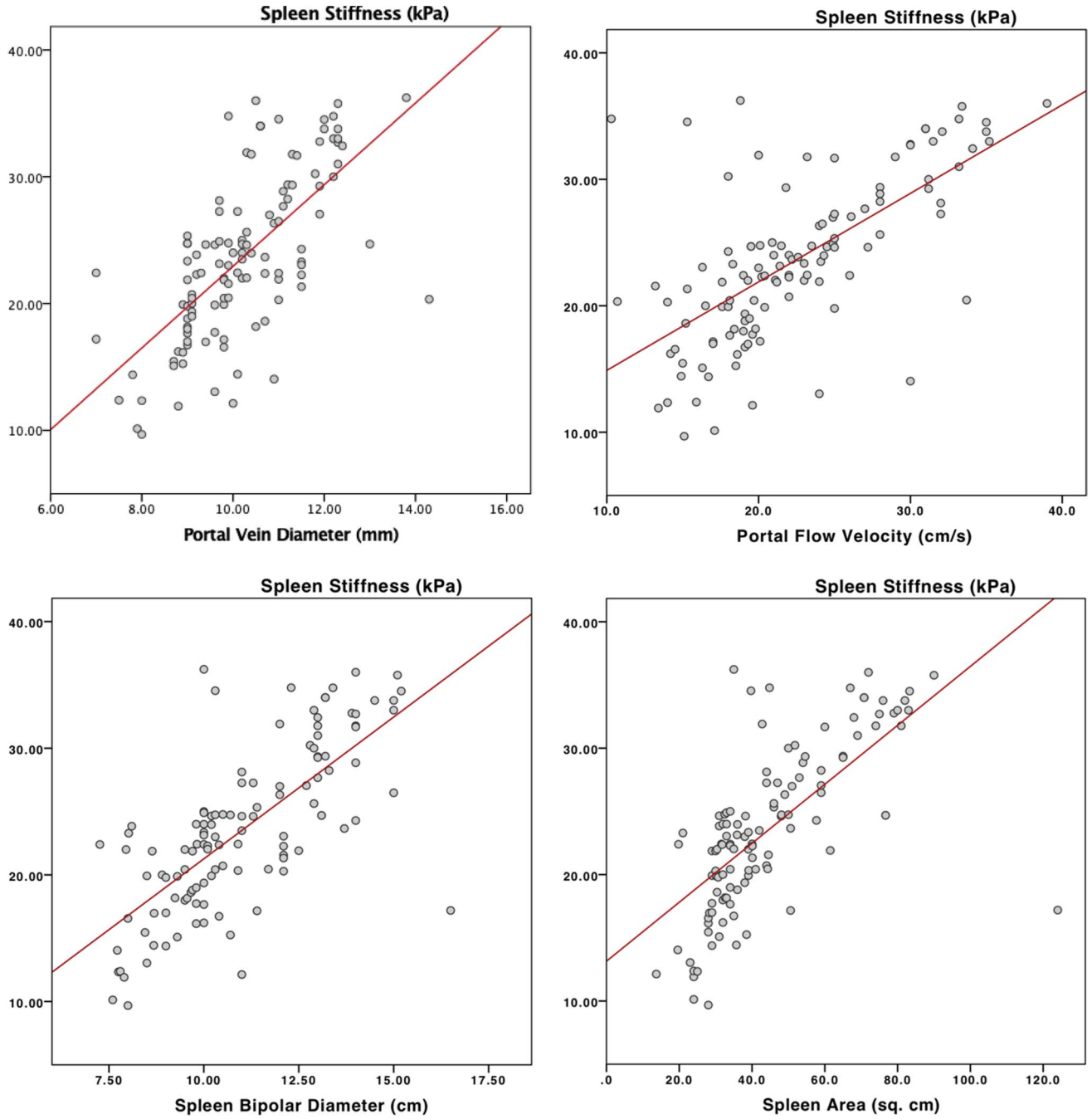

Fig. 1 Linear regression analyses between SS and spleno-portal axis parameters. Equations can be found in the main text. According to regression results, SS increased 3.220 kPa for each mm of portal vein diameter; 0.7 kPa for each cm/s of portal flow velocity; 2.239 kPa for each cm of spleen bipolar diameter; and 0.233 kPa for each cm^2 of splenic area

was found ($F(1,115) = 122.214$, $p < 0.001$), with an R^2 of 0.515. Patients' predicted SS is equal to [− 1.121 + 2.239 (spleen bipolar diameter)] kPa when spleen bipolar diameter is measured in cm. SS increased 2.239 for each of cm of spleen bipolar diameter. In terms of spleen area, a significant regression equation was found ($F(1,115) = 98.690$, $p < 0.001$), with an R^2 of 0.462. Patients' predicted SS is equal to [13.145 + 0.233 (spleen area)] kPa when spleen area is measured in cm^2. SS increased 0.233 for each cm^2 of spleen area. Multiple linear regression analysis was not feasible between the dependent variable (SS) and independent variables related to the spleno-portal axis due to multicollinearity since in the developed model $F(4;112) = 60.545$, with an R^2 of 0.684, variables showed the following VIFs: 1.647 (portal flow velocity), 5.597 (spleen bipolar diameter), and 5.164 (spleen area).

Spleen stiffness and ALT levels

As in Table 2, patients were stratified according to median ALT levels. Patients with higher ALT values (i.e., ALT > 32 IU/L) showed an increased median value of SS (28.5 vs. 19.2 kPa, $p < 0.001$), portal vein diameter (10.9 vs. 9.1 mm, $p < 0.001$), portal flow velocity (25.9 vs. 18.8 cm/s, $p < 0.001$), spleen bipolar diameter (12.8 vs. 9.75 cm, $p < 0.001$), and spleen area (54 vs. 32 cm^2, $p < 0.001$).

Furthermore, the relationship between SS and ALT levels follows a cubic polynomial form, which resulted in a significant regression equation ($F(3,113) = 257.921$, $p < 0.001$), with an R^2 of 0.873. Patients' predicted SS is equal to $[11.735 + 0.404\ (ALT)^1 - 0.002\ (ALT)^2 + 4.26 \times 10^{-6}\ (ALT)^3]$ kPa. As interpreting polynomial terms is complex, to visually evaluate the effect of ALT on SS, check the plot in Fig. 2.

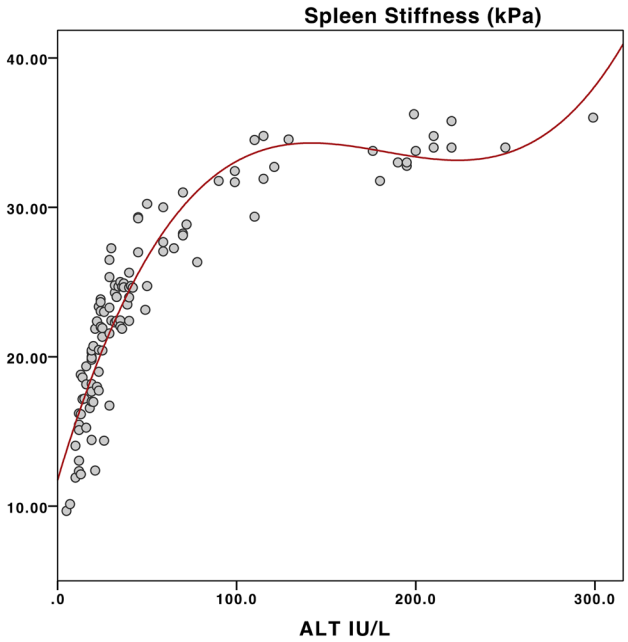

Fig. 2 Cubic polynomial regression between SS and serum ALT levels. The equation of predicted SS expressed in kPa is the following: $11.735 + 0.404\ (ALT)^1 - 0.002\ (ALT)^2 + 4.26 \times 10^{-6}\ (ALT)^3$

Discussion

Nowadays, spleen elastography is a hot topic in the hepatological community, especially for its role in esophageal varix screening. Therefore, it is appropriate to further study confounding factors that may affect SS values in the anticipation of future guidelines.

First, our results showed that patients with ongoing HCV infection and without significant liver fibrosis had higher SS ($p < 0.001$), portal vein diameter ($p = 0.031$), portal flow velocity ($p = 0.025$), spleen bipolar diameter ($p = 0.042$), spleen area ($p = 0.025$), and ALT levels ($p < 0.001$) when compared to healthy controls. Similar data, in terms of SS difference, have also been demonstrated in a comparable group of HCV patients and controls [29]. In addition, we had already demonstrated a weak positive correlation between SS and portal flow velocity and a weak negative correlation with the splenic area in a smaller sample of healthy individuals, which also showed up in the healthy controls of the current study [12]. The importance of the evaluation of SS in healthy subjects is

Table 2 Spleno-portal parameters and SS in patients affected by HCV stratified by median values and interquartile ranges of serum ALT

Variables	ALT < 32 (IU/L) $n = 58$	ALT > 32 (IU/L) $n = 59$	Significance
Spleen stiffness (kPa)	19.2 (16.2;21.9)	28.5 (25.6;32.7)	$p < 0.001$
Portal vein diameter (mm)	9.1 (8.9,10.1)	10.9 (10.6;12.9)	$p < 0.001$
Portal flow velocity (cm/s)	18.8 (16.3;20.8)	25.9 (21.2;31)	$p < 0.001$
Spleen bipolar diameter (cm)	9.75 (8.67;10.75)	12.8 (10.3;13.2)	$p < 0.001$
Spleen area (cm^2)	32 (29;39)	54 (38.2;70.8)	$p < 0.001$

Patients with ALT > 32 IU/L had higher spleen stiffness, portal vein diameter, portal flow velocity, spleen bipolar diameter, and spleen area

related to the already demonstrated overlap of stiffness values between healthy controls and cirrhotic patients without clinically significant portal hypertension [11, 30], thus highlighting the relevance of determining the confounding factors that may invalidate correct patient stratification. Therefore, we have tried to demonstrate through a linear regression model the effect of spleno-portal dynamics on stiffness values: SS increased by 3.220 kPa for each mm of portal vein diameter, by 0.7 kPa for each cm/s of portal flow velocity, by 2.239 kPa for each of cm of spleen bipolar diameter, and by 0.233 kPa for each cm^2 of spleen area.

These results want to serve as a proof-of-concept to stress the idea that SS should be adapted in light of other parameters that may affect its reliability. Because SS has been directly correlated to a hepatic vein pressure gradient (HVPG) [31–33], when this technique is not available, the hepatologist should be able to interpret SS to decide the appropriate follow-up.

Aside from portal hypertension stratification, liver disease centers are starting to measure SS in HCV patients undergoing DAA treatment serially, and some have observed that median SS values decrease after successful HCV eradication [17] and, particularly, the decrease in SS was higher in patients with higher ALT levels [34]. As a matter of fact, patients with higher ALT values showed an increased median value of SS (28.5 vs. 19.2 kPa), as well as spleno-portal axis parameters including portal flow velocity and splenic dimensions. These data may support the assumption that (1) inflammation affects SS values, (2) SS decrease after successful treatment may be related to reduced virus-induced cytolysis, which may have induced a momentary and reversible state of inflammation-induced portal congestion [35].

In conclusion, the results of our study may become a starting point for further investigations into the role of inflammation as a confounding factor in SS evaluation, and can perhaps increase the accuracy of spleen elastography in diagnostic and follow-up procedures.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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