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Modified spleen stiffness measurement by transient elastography is associated with presence of large oesophageal varices in patients with compensated hepatitis C virus cirrhosis

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SUMMARY. To evaluate the accuracy of liver transient elastography (TE), spleen TE and other noninvasive tests (AAR, APRI score, platelet count, platelet/spleen ratio) in predicting the presence and the size of oesophageal varices in compensated hepatitis C virus (HCV) cirrhosis, we studied 112 consecutive patients with compensated HCV cirrhosis who underwent biochemical tests, gastrointestinal endoscopy, liver TE and spleen TE by Fibroscan® (Echosens, Paris, France) using a modified software version with a range between 1.5 and 150 kPa. Spleen TE was not reliable in 16 patients (14.3%). Among the 96 patients with a valid measurement (69.8% men, mean age: 63.2 ± 9.5 years), 43.7% had no oesophageal varices, 29.2% had grade 1% and 27.1% had grade 2 or grade 3 oesophageal varices. Patients with values of 75 kPa by standard spleen TE had mean values of modified spleen TE of 117 kPa (range: 81.7–149.5). Linear regression revealed a significant correlation between modified spleen TE and oesophageal varix size ($r = 0.501$; beta: 0.763, SE: 0.144; $P < 0.001$). On univariate analysis, the variables associated with grade 2/grade 3 oesophageal varices were AAR score, APRI score, platelet/spleen ratio, liver TE and modified spleen TE. On multivariate analysis, only modified spleen TE (OR: 1.026; 95% CI: 1.007–1.046; $P = 0.006$) and AAR (OR: 14.725; 95% CI: 1.928–112.459; $P = 0.010$) remained independently associated with grade 2/grade 3 oesophageal varices. Platelet/spleen ratio was the best predictor of oesophageal varices area under the ROC curve (AUROC: 0.763, cut-off: 800, sensitivity: 74%, specificity: 70%), while modified spleen TE was more accurate in predicting grade 2/grade 3 oesophageal varices (AUROC: 0.82, cut-off: 54.0 kPa, sensitivity: 80%, specificity: 70%). Portal hypertension increases spleen stiffness, and the measurement of modified spleen TE is an accurate, noninvasive tool for predicting the presence of large oesophageal varices in patients with compensated HCV cirrhosis.

Keywords: cirrhosis, liver stiffness, oesophageal varices, spleen stiffness, transient elastography.

INTRODUCTION

The diagnosis of cirrhosis and portal hypertension is the principal decision-making point to consider in patients with chronic liver disease due to hepatitis C virus (HCV) infection [1]. Although liver biopsy is the mainstay for the assessment of fibrosis and the diagnosis of cirrhosis [2], and evaluation of oesophageal varices (EV) is made by endoscopy [3,4], noninvasive methods could conceivably replace these procedures [5–8]. Several studies [9–11] and two meta-analyses [12,13] have shown that liver stiffness measurement by transient elastography (TE) is the most accurate noninvasive method for detecting cirrhosis in patients with chronic HCV hepatitis. However, when liver TE was used to indirectly evaluate the degree of portal hypertension and the likelihood of presence of EV [11,14–16], the results were less satisfactory. Similarly, the platelet count [17], the measurement of the spleen diameter by ultrasound scan (US) [18] and, in particular, the platelet count/spleen diameter ratio [19,20], while having been identified as noninvasive methods to classify patients with cirrhosis and EV, still lack in sensitivity and specificity.

In patients with cirrhosis, splenomegaly is caused both by portal congestion, and tissue hyperplasia and fibrosis.

Abbreviations: EV, oesophageal varices; HCV, hepatitis C Virus; LS, liver stiffness; ROC, receiver operating characteristic curves; SS, spleen stiffness; TE, transient elastography; US, ultrasound scan.

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Histological observation of the spleen in patients with cirrhosis shows a pooling of blood in the red pulp of the spleen, hyperplasia of histiocytes and myofibroblasts, and an increase in reticular fibres and hyperplasia of arterials [21]. The increase in spleen size is followed by an increase in splenic blood flow, which actively participates in portal hypertension by congesting the portal system [22]. The rapid decrease in outflow resistance of the splenic vein, followed by a slow decrease in spleen size and by an increase in the platelet count after liver transplantation, confirms that both splenic haemodynamics and splenomegaly are caused by congestion and tissue hyperplasia [23,24].

The hypothesis of our study was that spleen stiffness measurement by TE could conceivably be a noninvasive measure of portal hypertension and the likelihood of the presence of oesophageal varices. With this aim, we planned a prospective observational study that included patients with compensated liver cirrhosis secondary to HCV to evaluate spleen TE as a diagnostic tool.

PATIENTS AND METHODS

Patients

In this prospective study, we included patients with a new clinical or histological diagnosis of HCV Child-Pugh A cirrhosis and who underwent gastrointestinal endoscopy (GIE) as screening for EV. All patients had a serum HCV RNA positivity by PCR (Roche TaqMan HCV ver 2.0, Basel, Switzerland). We excluded patients with HIV and/or HBV co-infection, history of alcohol abuse (≥ 20 g/day in the last year or more, evaluated by questionnaire) or previous liver decompensation and/or variceal bleeding, diagnosis of hepatocellular carcinoma (HCC) and/or portal thrombosis, and patients on treatment with β-blockers. The study was carried out in accordance with the principles of the Declaration of Helsinki and approved by our hospital's Ethics Committee. All patients gave written informed consent to perform TE of the liver and the spleen as an extra clinical procedure.

Procedures

All patients included in the study underwent single-day haematological and biochemical tests, abdominal ultrasound scan (US) examination, liver and spleen TE, and GIE. Haematological (platelet count, leucocyte count and haemoglobin levels) and liver function (bilirubin, albumin, AST/ ALT levels and prothrombin time) tests were conducted to evaluate the Child-Pugh score, the AST/ALT ratio (AAR), the AST-to-platelet ratio index (APRI) and the platelet count/spleen diameter ratio. US and TE were performed by FibroScan™ (Echosens, Paris, France) after overnight fasting. By US, we evaluated liver structure to exclude patients with HCC and the presence of ascites. We also measured the longitudinal diameter of the spleen. Liver stiffness (LS) was evaluated in the right lobe of the liver through intercostal spaces, with the patient in the supine position and the right arm in maximal abduction [25]. The longitudinal diameter of the spleen was measured by US with the patient in the supine position and the left arm in maximal abduction. Under US guidance, the axis of the FibroScan™ probe was placed perpendicular to the plane of the thorax in the intercostal space corresponding to the spleen parenchyma. In all patients, at least 10 measurements of LS and spleen stiffness (SS) were taken. The success rate was calculated as the ratio of the number of successful measurements to the total number of acquisitions. Median values of the successful measurements were kept as representative of LS and SS, and results were expressed in kilopascal (kPa). Only examinations with at least 10 valid measurements and a success rate of more than 60% were considered reliable. TE of the liver and the spleen was performed by two expert physicians (V.C., F.B.). To assess interobserver variability, 15 patients selected at random were evaluated by both operators, each of whom was blinded to the results obtained by the other. The concordance coefficient between interobserver evaluations was 0.97. STE was analysed first with standard FibroScan™ software and subsequently with a modified software version, not commercially available, and provided by Echosens, which allows measurement of stiffness between 1.5 and 150 kPa, defined as modified spleen stiffness (mSS). GIE was performed in all patients by a single operator (F.S.), and oesophageal varices were graded according to the North Italian Endoscopic Club for the Study and Treatment of Esophageal Varices [26]. EV were classified as small (G1), when they occupied less than 30% of the lumen; medium (G2), when occupying between 30% and 60% of the lumen; and large (G3), if more than 60% of the lumen was occupied.

Statistics

Continuous variables were summarized as mean ± SD and categorical variables as frequency and percentage. Multiple logistic regression models were used to assess the relationship between demographic, biochemical and instrumental data and to detect the presence of EV. We selected age, gender, AST/ALT ratio (AAR), AST-to-platelet ratio index (APRI), platelet count, spleen diameter, platelet count/spleen diameter ratio, LS, SS and mSS as noninvasive variables associated with the presence of EV and/or large EV. Variables found to be associated with the dependent variables on univariate logistic regression with a probability threshold of <0.05 were included in the multivariate logistic regression models. Regression analysis was performed using the SPSS statistical package (version 15.0; SPSS Inc, Chicago, IL, USA). Using MedCalc statistical software, we obtained the receiver operating characteristic (ROC) curves

and the best cut-off for identifying the area under the ROC curve (AUROC) of LTE and STE for predicting the presence of EV and large EV in patients with compensated cirrhosis. Sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV and NPV), positive likelihood ratio (+LR) and negative likelihood ratio (−LR) were calculated using cut-offs resulting in a diagnosis of compensated cirrhosis with EV.

RESULTS

Patient features

One hundred and twelve patients with clinical or histological diagnosis of compensated cirrhosis were included in the study between January 2008 and March 2011. In 16 cases (14.3%), TE of the spleen was not conclusive, and these patients were excluded from the study. In six of them, all with a body mass index (BMI) ≥ 30, also TE of the liver could not be performed (5.3% of the whole cohort). Ninety-six patients were analysed. Sixty-seven of them (69.8%) were men, the mean age was 63.2 ± 9.5 years, and the mean BMI was 27.0 ± 3.0 kg/sm. The mean values of albumin, bilirubin and INR were 4.0 ± 0.3 g/dL, 1.1 ± 0.6 mg/dL and 1.0 ± 0.1, respectively. Seventy-six patients had a Child-Pugh score (CPS) of 5, and 20 patients a CPS of 6. EV were found in 54 patients (56.3%), with 28 patients (29.2%) having grade 1 EV, 17 patients (17.7%) having grade 2 and 9 patients (9.4%) having grade 3.

Standard spleen stiffness in the evaluation of oesophageal varices

The mean spleen diameter was significantly lower in the 16 patients without a reliable SS measurement compared with the 96 patients with a reliable spleen stiffness evaluation (12.3 ± 2.4 cm *vs* 14.6 ± 2.9 cm; $P = 0.005$). The mean SS value for the entire cohort was 48.7 ± 20.6 kPa. In 21 cases (21.7%), the SS value was equal to 75 kPa. As shown in Table 1, we observed a significant difference in mean SS values between patients without EV and those with EV (44.7 ± 17.9 kPa *vs* 55.6 ± 19.5 kPa; $P = 0.006$). Similarly, as shown in Table 3, the mean values of SS were significantly lower in patients without EV or with grade 1 EV than the values observed in patients with grade 2 or grade 3 EV (45.3 ± 18.7 kPa *vs* 64.7 ± 13.6 kPa; $P < 0.001$).

Modified spleen stiffness in the evaluation of oesophageal varices

All SS measurements were then analysed by Echosens, using a software version that allows measurement of stiffness between 1.5 and 150 kPa. Figure 1 shows the distri-

Table 1 Clinical and biochemical features of 96 patients with compensated HCV cirrhosis according to the presence of EV

	Cirrhosis without EV (42 patents)	Cirrhosis with EV (54 patients)	*P* value	Multivariate analysis	
				OR (95% CI)	*P* value
Gender (Male,%)	28 (66.7)	39 (72.0)	0.566	–	–
Age (years, mean, SD)	60.7 ± 10.5	65.1 ± 8.2	0.122	–	–
AST (U/L, mean, SD)	89.0 ± 63.4	89.5 ± 74.8	0.970	–	–
ALT (U/L, mean, SD)	120.3 ± 91.3	93.1 ± 60.4	0.101	–	–
AST/ALT ratio	0.78 ± 0.25	1.02 ± 0.33	<0.001	7.363 (1.182–45.844)	0.032
PLT ($\times 10^3$/mL)	128.5 ± 41.8	89.6 ± 35.5	<0.001	–	–
APRI test	1.86 ± 1.36	2.73 ± 2.00	0.017	0.989 (0.680–1.439)	0.954
Spleen diameter (cm)	14.1 ± 3.1	15.0 ± 2.8	0.157	–	–
PLT count /spleen diameter ratio	981.3 ± 390.6	628.1 ± 278.9	<0.001	0.998 (0.996–0.999)	0.006
Liver Stiffness (kPa, mean, SD)	17.9 ± 9.4	25.7 ± 14.0	0.002	1.012 (0.956–1.072)	0.678
SR (%, mean, SD)	85.7 ± 17.6	85.3 ± 24.6	0.935		
IQR (mean, SD)	3.4 ± 2.0	4.6 ± 4.5	0.101		
Spleen Stiffness (kPa, mean, SD)	44.4 ± 18.4	55.3 ± 19.6	0.007	–	–
SR (%, mean, SD)	82.8 ± 15.1	82.7 ± 15.2	0.968	–	–
IQR (mean, SD)	9.7 ± 7.8	8.9 ± 8.9	0.624	–	–
Modified Spleen Stiffness (kPa, mean, SD)	48.4 ± 26.3	69.4 ± 39.5	0.002	1.011 (0.994–1.029)	0.214
SR (%, mean, SD)	82.8 ± 15.1	82.7 ± 15.2	0.968		
IQR (mean, SD)	17.1 ± 14.5	18.7 ± 12.2	0.559		

AST, aspartate aminotransferase; ALT, alanine aminotransferase; EV, oesophageal varices; HCV, hepatitis C virus; IU, International units; INR, international normalized ratio; PLT, platelets. SD, standard deviation.

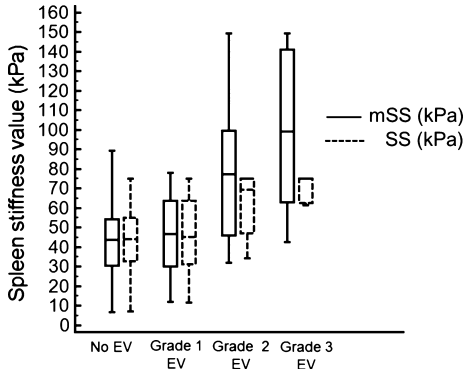

Fig. 1 Distribution of spleen stiffness and median modified spleen stiffness values calculated for patients with compensated hepatitis C virus cirrhosis at different grades of oesophageal varices.

bution of standard SS and mSS mean values calculated for patients with compensated HCV cirrhosis with different EV grades. All 21 patients (21.9%) who had values of 75 kPa by standard TE had a mean mSS value of 118 KPa, with a range from 81.7 to 149.5 kPa. The rate of patients with EV (50.7% *vs* 76%, $P = 0.028$) and with grade 2 or grade 3 EV (19.7% *vs* 56% $P = 0.001$) was significantly different between patients with an mSS lower or higher than 75 KPa. Furthermore, on linear regression analysis, we found a higher regression coefficient between EV size and mSS ($r = 0.501$; beta: 0.763, SE: 0.144, $P < 0.001$) than between EV size and SS ($r = 0.437$; beta 0.382, SE 0.081 $P < 0.001$). Taking into account the higher correlation coefficient of mSS with EV, we decided to use this instead of the SS for analysis of the data.

Noninvasive serum fibrosis tests, liver stiffness and modified spleen stiffness in predicting the presence of oesophageal varices

Patients with EV had a significantly higher value of AST/ALT ratio, platelet count, APRI, spleen diameter, PLT count/spleen diameter ratio, LS and mSS on univariate analysis (Table 1). On multivariate analysis, only AAR (OR: 7.363; 95% CI, 1.182–45.884; $P = 0.032$) and PLT count/spleen diameter ratio (OR: 0.998; 95% CI, 0.996–0.999; $P = 0.006$) were independently associated with the presence of EV (Table 1). ROC curve analysis identified the PLT/Spleen ratio as the noninvasive test of fibrosis that achieved the best performance for predicting EV (AUROC: 0.763, cut-off: 800, sensitivity: 74%, specificity: 70%, PPV: 77%, NPV: 67%). The best cut-off value of modified SS for predicting EV was ≥ 50.0 kPa (AUROC 0.70, sensitivity: 65%, specificity: 61%, PPV: 69%, NPV: 57%; Table 2).

Noninvasive serum fibrosis tests, liver stiffness and modified spleen stiffness in predicting the presence of large oesophageal varices

Similarly, we performed a univariate analysis to identify the clinical and biochemical variables associated with the presence of grade 2 or grade 3 varices in patients with compensated HCV cirrhosis. As expected, the difference in mean AST/ALT ratio, platelet count, APRI, spleen diameter, PLT count/spleen diameter ratio, LS and mSS were found to be statistically significant between patients without EV or with small EV and patients with large varices (Table 3). On multivariate analysis, the independent predictors of grade 2 or grade 3 varices were AAR (OR: 14.725, 95% CI: 1.928–112.459; $P = 0.010$) and mSS (OR: 1.026, 95% CI: 1.007–1.046; $P = 0.006$) only (Table 3). On AUROC analysis, mSS was the more accurate tool for predicting grade 2 or grade 3 EV (AUROC: 0.82, cut-off: 54.0 kPa, sensitivity: 80%, specificity: 70%, PPV: 47%, NPV: 90%; Table 4).

DISCUSSION

Various studies [9–11] and two meta-analyses [12,13] assessing the diagnostic accuracy of liver TE in staging fibrosis in patients with chronic hepatitis C have reported

Table 2 Performances of liver stiffness and spleen stiffness in predicting EV in 96 patients with compensated HCV-related liver cirrhosis

Prevalence 54/96 (56.3%)	AUROC	Cut-off	SE	95% CI	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	+LR	− LR	Correctly classified (%)
LS (kPa)	0.707	17.0	0.054	0.60–0.80	71	57	67	62	1.7	0.5	60 (62.5)
Modified SS	0.701	50.0	0.056	0.57–0.77	65	61	69	57	1.7	0.6	60 (62.5)
AAR	0.732	0.80	0.051	0.63–0.82	69	67	73	62	2.1	0.5	66 (69)
APRI	0.657	1.5	0.056	0.55–0.75	67	52	64	55	1.6	0.6	58 (60.4)
PLT/Spleen	0.763	800.0	0.051	0.66–0.84	74	70	77	67	2.5	0.4	68 (71)

AUROC, area under the ROC curve, EV, oesophageal varices; HCV, hepatitis C virus; LR, likelihood ratio; LS, liver stiffness; NPV, negative predictive values; PPV, positive predictive values.

Table 3 Clinical and biochemical features of 96 patients with compensated HCV cirrhosis according to the presence of large EV

	No EV, Small EV (70 patents)	Large EV (26 patients)	*P* value	Multivariate analysis	
				OR (95% CI)	*P* value
Gender (Male, %)	47 (67.1)	20 (76.9)	0.354	–	–
Age (years, mean, SD)	62.5 ± 9.4	64.9 ± 9.7	0.275	–	–
AST (U/L, mean, SD)	87.4 ± 58.4	94.3 ± 94.7	0.670	–	–
ALT (U/L, mean, SD)	111.9 ± 80.6	86.6 ± 60.8	0.150	–	–
AST/ALT ratio	0.84 ± 0.27	1.13 ± 0.34	<0.001	14.725 (1.928–112.459)	0.010
PLT ($\times 10^3$/mL)	114.7 ± 41.9	85.0 ± 38.1	0.002	–	–
APRI	2.13 ± 1.50	2.95 ± 2.35	0.045	1.38 (0.731–1.474)	0.834
Spleen diameter (cm)	14.1 ± 2.9	15.8 ± 2.9	0.011	0.770 (0.480–1.236)	0.280
PLT count /spleen diameter ratio	865.2 ± 377.1	555.8 ± 257.4	<0.001	0.992 (0.981–1.004)	0.211
Liver stiffness (kPa, mean, SD)	20.0 ± 10.7	28.2 ± 15.7	0.005	0.990 (0.944–1.037)	0.663
SR (%, mean, SD)	86.7 ± 21.5	82.2 ± 22.4	0.372		
IQR (mean, SD)	3.5 ± 2.5	5.5 ± 5.4	0.100		
Spleen stiffness (kPa, mean, SD)	45.3 ± 19.0	64.6 ± 14.1	<0.001	–	–
SR (%, mean, SD)	82.7 ± 15.8	83.0 ± 13.2	0.930	–	–
IQR (mean, SD)	9.6 ± 7.5	8.3 ± 10.5	0.556	–	–
Modified spleen stiffness (kPa, mean, SD)	50.7 ± 30.0	85.8 ± 38.1	<0.001	1.026 (1.007–1.046)	0.006
SR (%, mean, SD)	82.7 ± 15.8	83.0 ± 13.2	0.930		
IQR (mean, SD)	16.7 ± 13.6	21.4 ± 11.6	0.120		

AST, aspartate aminotransferase; ALT, alanine aminotransferase; EV, oesophageal varices; HCV, hepatitis C virus; IU, International units; INR, international normalized ratio; PLT, platelets; SD, standard deviation.

Table 4 Performances of liver stiffness and spleen stiffness in predicting large EV in 96 patients with compensated HCV-related liver cirrhosis

Prevalence 26/96 (27.1%)	AUROC	Cut-off (kPa)	SE	95% CI	Sens. (%)	Spec. (%)	PPV (%)	NPV (%)	+LR	− LR	Correctly classified (%)
LS (kPa)	0.710	19.0	0.060	0.61–0.80	72	55	38	84	1.6	0.5	54 (56.2)
Modified SS	0.819	54.0	0.053	0.70–0.86	80	70	47	90	2.0	0.3	66 (68.9)
AAR	0.751	1.0	0.058	0.65–0.83	69	72	49	86	2.5	0.4	65 (67.7)
APRI	0.631	2.0	0.064	0.53–0.73	60	56	33	78	1.3	0.7	55 (57.3)
PLT/Spleen	0.740	640	0.054	0.64–0.83	73	65	44	86	2.1	0.4	64 (66.7)

AUROC, area under the ROC curve; EV, oesophageal varices; HCV, hepatitis C virus; LR, likelihood ratio; LS, liver stiffness; NPV, negative predictive values; PPV, positive predictive values.

an NPV higher than 90% for the diagnosis of cirrhosis, suggesting greater accuracy in excluding than in confirming it, although liver TE is inaccurate in the assessment of portal hypertension [11,14–16,27]. Although Kazemi *et al.* [16] demonstrated that an LS $\geq$19 kPa had a 93% NPV for the presence of large varices, suggesting a potential role for TE in selecting patients for endoscopic screening, a study by Vizzuti *et al.* [14], while finding a solid correlation between LS and the hepatic venous pressure gradient (HVPG), rated TE quite poorly in predicting and grading EV, based on a negative predictive value of 66%. In a study by Bureau *et al.* [15], LS correlated with the presence of EV, although no association between LS values and the size of EV was found. Castera *et al.* [11] reported the optimized cut-offs for the detection of EV at 21.5 kPa (73% of patients correctly classified) and large EV at 30.5 kPa (79% of patients correctly classified). However, they found that several blood markers (platelets, AST/ALT ratio, FibroTest, prothrombin index, Lok index) performed better than TE for predicting both EV and large EV. Finally, in a recent study [28], our group reported that in patients with compensated HCV cirrhosis, the platelet/spleen ratio and

insulin resistance measured by HOMA-IR, regardless of the presence of diabetes, significantly predicted the presence of EV, outweighing the contribution offered by TE.

We hypothesized that the measurement of spleen stiffness by TE could be a noninvasive method for evaluating the presence of EV. To determine whether SS could conceivably be a noninvasive measure capable of evaluating the likelihood of presence of EV, we carried out this prospective observational study, including consecutive patients with compensated HCV cirrhosis evaluated for the presence of oesophageal varices. This possibility was already suggested by Stefanescu *et al.* [29] in a study describing the correlation between SS values and the presence of EV and more recently by Colecchia *et al.* [30] who evaluated 113 patients with a diagnosis of liver cirrhosis. Similar to this study, we enrolled 112 patients with newly diagnosed compensated HCV cirrhosis who had measurement of liver and spleen stiffness on the same day as GIE. The rate of SS measurement failure is similar in the two studies (14% *vs* 11%).This was due to a high BMI in some, but more frequently due to a success rate of measurement of less than 60%. SS measurement failed less frequently in patients with larger measurable volume of the spleen. In fact, it was easier to localize, under US guidance, the middle of the region of interest (ROI) in a spleen with a longitudinal diameter of at least 12 cm and a mean anteroposterior diameter of 4 cm. In addition, the prevalence of EV was analogous (54% *vs* 53%) in the two studies, and similar cut-off values for diagnosis of EV were reported. However, Colecchia *et al.* [30] found an overall higher performance of TE of the spleen for diagnosis of EV compared with our study. The higher age of our cirrhotic patients with a more stabilized portal hypertension demonstrated by a larger spleen diameter and lower platelets count could be associated with the development of many portosystemic collateral shunts. This may represent a possible explanation of the different performance of SS in diagnosing the presence of EV. Further studies evaluating the role of the spleen stiffness in diagnosing portal hypertension may clarify better this issue. Furthermore, Colecchia *et al.* [30] did not analyse the performance of noninvasive tests for diagnosis of large varices. Indeed, Baveno guidelines recommend a primary prophylaxis with beta-blockers or variceal band ligation in these patients, and defining the role of spleen stiffness for diagnosis of large EV could be relevant in the management of patients with liver cirrhosis. Actually, in our cohort of patients, mSS has been the more accurate tool for predicting grade 2 or grade 3 EV with an AUROC of 0.82 and a NPV of 90%.

Furthermore, given our high rate of patients with an SS value ≥ 75 kPa, we asked Echosens to revise our spleen stiffness results to confirm the data and to evaluate the exact range of values of the spleen stiffness. To overcome a potential ceiling effect, Echosens performed an analysis of the data using software that allows measurement of a stiffness between 1.5 and 150 kPa.

Our study, in addition to the assessment of LS and SS, included, in parallel, an analysis of the performance of such noninvasive tests as the AST/ALT ratio, APRI test and platelet count/spleen diameter ratio in predicting cirrhosis with EV. Previous studies have concluded that LS cannot replace endoscopy as screening for EV in patients with cirrhosis [11,14,15]. In our study, LS was confirmed as having a modest sensitivity and specificity in discriminating cirrhotic patients with EV, because, as in other studies, the test correctly identified less than 65% of patients.

In addition, mSS performed more poorly than PLT/spleen count and AAR in diagnosing EV, although when we analysed the performances of the noninvasive tool in diagnosing the presence of grade 2 or grade 3 EV, mSS performed better. The best cut-off of mSS for predicting the presence of large EV was set by ROC curve at ≥ 54 kPa and obtained an AUROC of 0.82 and an NPV of 90%, suggesting that in patients with cirrhosis, the performance of mSS in predicting the presence of grade 2 or 3 EV was clearly better than that of LS and also better than any other biochemical or instrumental variable linked to portal hypertension, such as longitudinal spleen diameter, platelets and even the platelet count/spleen diameter ratio, which is considered the most sensitive predictor of EV in this setting [19,20].

We have found an high odds ratio of AAR in predicting both EV and large EV. This datum is not completely new. Indeed, Casterà *et al.* [11] have shown that the ability of AAR in diagnosing EV was higher than TE of the liver in their cohort of patients with cirrhosis. However, Colecchia *et al.* [30] did not report the performance of AAR in their study. Further studies are needed to address this issue.

This study has same limitations. First, we used a FibroScan™ probe validated only for the measurement of LS. Indeed, the acquisition parameters of the Fibroscan® were optimized for stiffness assessment of liver tissues, especially in terms of low-frequency excitation. To accurately assess the stiffness of organs harder than the liver, the acquisition parameters (low-frequency excitation, pulse repetition frequency) should be modified. Normal spleen stiffness is reported to be higher than that of the liver. Therefore, despite the use of a modified algorithm, the use of the Fibroscan® on the spleen of patients with cirrhosis might lead to overestimated stiffness values. The vascular structure and the small volume of the spleen could be a limitation for the measurement of spleen stiffness. Furthermore, presence of ascites represents another important limitation for these patients. Indeed, it is not unusual that we need to diagnose the oesophageal varices in patients with decompensated cirrhosis. However, in collaboration with Echosens, which allowed an extension of the upper limit of measurement, we were able to obviate the problem of a

high rate of spleen stiffness values ≥ 75 kPa, which is the upper value of the measure scale of the FibroScan™. This allowed us to identify the correct range of spleen stiffness values and discover that FibroScan™ can be a useful tool for measuring it. Another clinically important issue that needs to be assessed in the future is the evaluation of the impact of beta-blockers on the mSS. The use of mSS in this setting could avoid the repetition of the portosystemic gradient measurement, reducing the costs and the discomfort of patients.

In conclusion, our data suggest that mSS can help clinicians in identifying compensated cirrhosis with clinically significant portal hypertension. After external validation in large, independent settings in a larger number of patients with cirrhosis of various aetiologies, these data might lead to avoidance of universal endoscopic screening.

AUTHORS' CONTRIBUTIONS

V. Di Marco, V.Calvaruso and A. Craxì were involved in the study concept and design; V. Calvaruso, F. Bronte, E. Conte and F. Simone contributed to the acquisition of data; V. Calvaruso and V. Di Marco were involved in analysis and data interpretation; V.Calvaruso and V. Di Marco drafted the manuscript; V. Di Marco and A, Craxì involved in the critical revision of the manuscript; V. Calvaruso performed statistical analysis.

CONFLICT OF INTERESTS

None.

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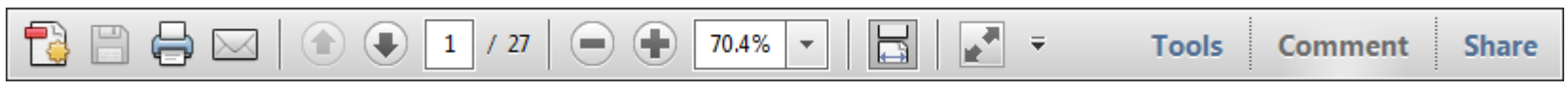
Required software to e-Annotate PDFs: Adobe Acrobat Professional or Adobe Reader (version 7.0 or above). (Note that this document uses screenshots from Adobe Reader X)
The latest version of Acrobat Reader can be downloaded for free at: http://get.adobe.com/uk/reader/

Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:



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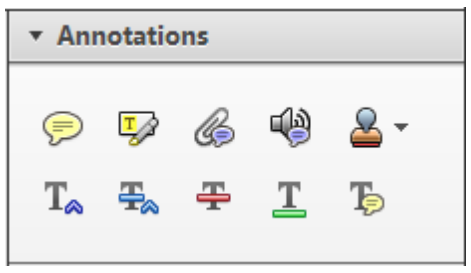
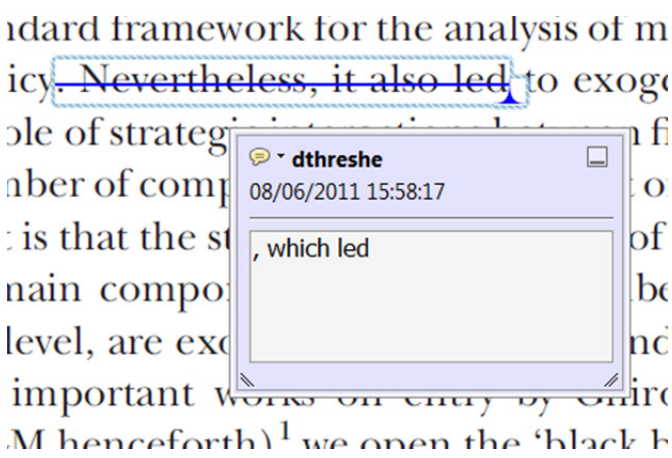


1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the Replace (Ins) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

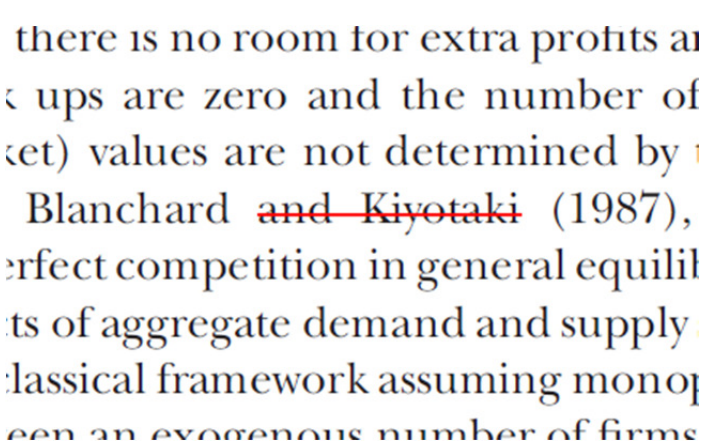


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.



3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
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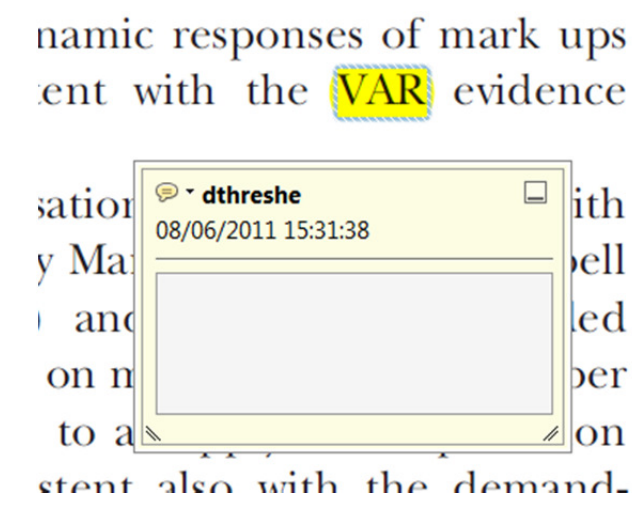
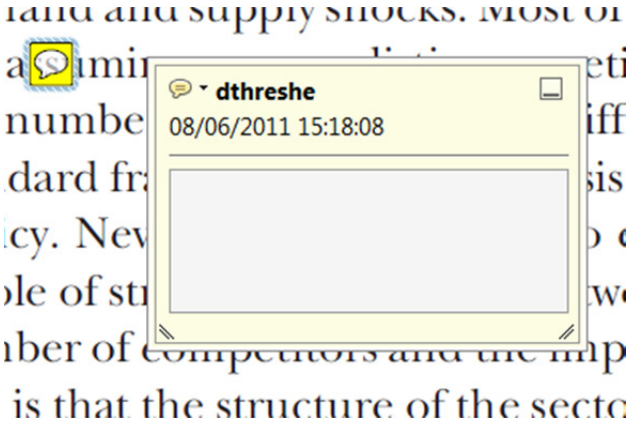


4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.



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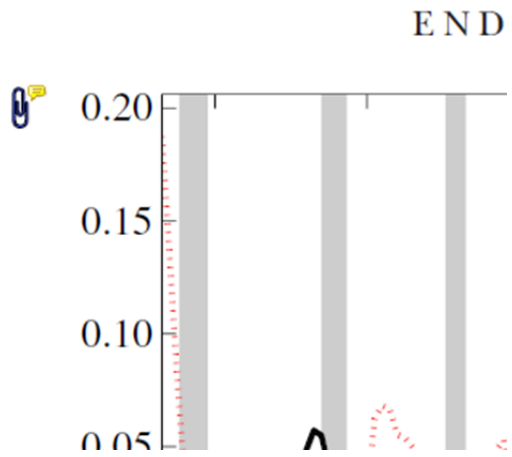
5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.



6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it

- Click on the Add stamp icon in the Annotations section.
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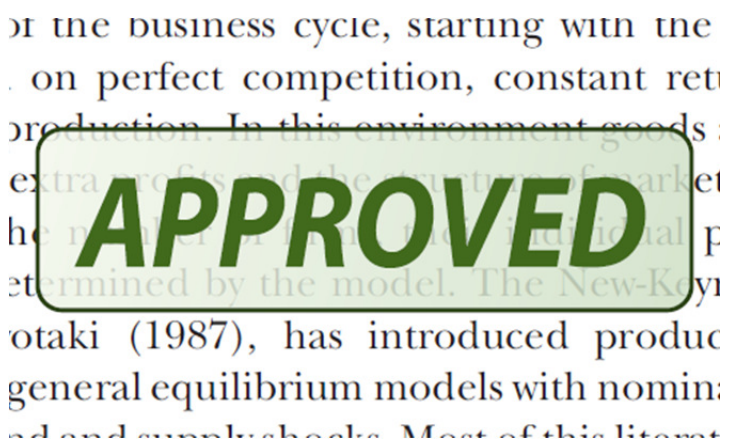
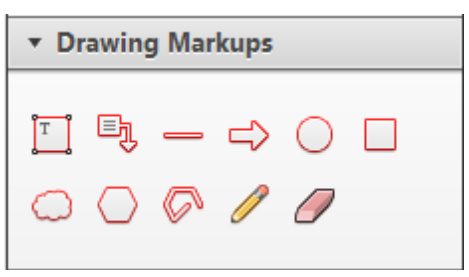


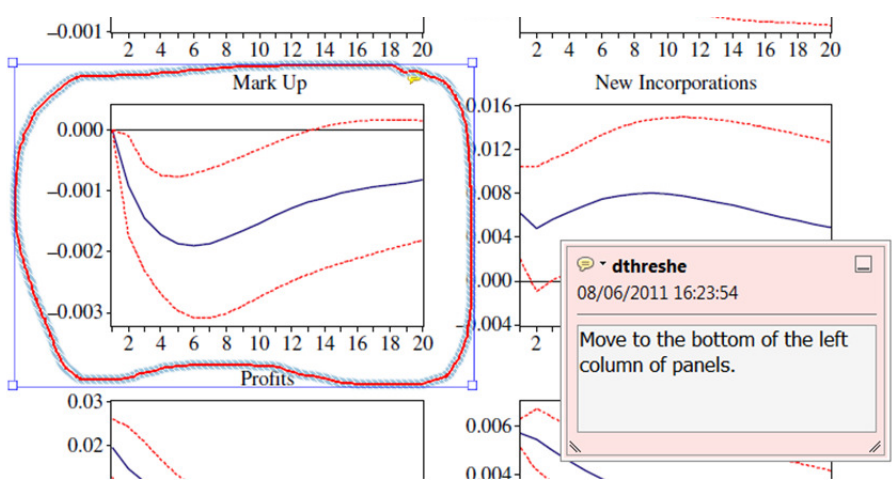


7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



For further information on how to annotate proofs, click on the Help menu to reveal a list of further options: