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Ultrasonographic evaluation of liver surface and transient elastography in clinically doubtful cirrhosis☆

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Background & Aims: Both transient elastography (TE) and left lobe liver surface (LLS) ultrasound may non-invasively detect cirrhosis (LC). We aimed to examine the diagnostic value of these methods in patients with a suspicion but not a definite diagnosis of cirrhosis.

Methods: We enrolled 90 patients with clinical suspicion of cirrhosis and a strong co-existing differential diagnosis requiring further invasive evaluation. They underwent hepatic venous pressure gradient (HVPG) measurement ± transjugular liver biopsy, LLS and TE. Images of LLS were digitally post-processed to obtain a numerical value (quantitative LLS, qLLS). TE < 12 kPa was considered to exclude LC, ⩾18 kPa diagnosed LC, and 12–18 kPa indeterminate. Technical failures were considered 'indeterminate'. Diagnosis of cirrhosis was confirmed by histology (84%) or by clinical data and HVPG ⩾ 10 mm Hg. Diagnostic accuracy was evaluated by positive and negative likelihood ratios (+LR and −LR).

Results: Cirrhosis was diagnosed in 44 patients. There were 14 technical failures with TE and 1 with LLS ($p = 0.001$). TE and LLS had similar diagnostic accuracy but gave complementary information: TE was mildly more accurate than LLS to rule out LC (−LR: 0.08 vs. 0.10), while it was less accurate to rule it in (+LR 5.05 vs. 11.15). Their combination offered the best diagnostic performance (+LR 9.15; −LR 0.06).

Conclusions: LLS is more technically applicable than TE. In patients with clinical suspicion of cirrhosis, LLS is the best non-invasive method to diagnose cirrhosis, while TE is preferable to rule it out. The combination of both holds the best diagnostic accuracy.

Keywords: Liver cirrhosis; Liver biopsy; HVPG; Ultrasound.
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Abbreviations: LC, liver cirrhosis; TE, transient elastography; LLS, left lobe liver surface; AUROC, area under the receiver operating characteristic curve; HVPG, hepatic venous pressure gradient; TJLB, transjugular liver biopsy.

Introduction

Liver cirrhosis is defined by anatomical changes within the liver parenchyma, including fibrosis and the development of regenerating nodules [1,2]. The appearance of cirrhosis is a hallmark in the natural history of chronic liver diseases, such as alcoholic HCV or HBV infection, because it identifies the point to initiate surveillance for hepatocellular carcinoma [3] and oesophageal varices [4]. Additionally, in alcoholic patients it is important to differentiate cirrhosis from alcoholic hepatitis, due to the different management and prognosis of these conditions [5,6].

Although liver biopsy is considered the gold standard for the diagnosis of cirrhosis, it has important limitations, such as being invasive, having the potential for complications and false-negative results or underestimating disease severity due to sampling error [7–9].

In recent years, many studies have evaluated the possibility of diagnosing cirrhosis by non-invasive methods. Transient elastography (TE) estimates liver stiffness, which is thought to be mainly determined by fibrosis, and is proven to be accurate for the non-invasive diagnosis of severe fibrosis and cirrhosis [10]. However, technical limitations preclude the use of TE in obese patients and in patients with ascites, and liver stiffness is increased by hepatic necroinflammation, cholestasis and increased central venous pressure. Moreover, the cut-off of liver stiffness for the identification of cirrhosis appears to be different in different aetiologies of liver disease and has not yet been evaluated in all comers with clinical suspicion of cirrhosis.

Ultrasound is usually the first imaging method used in patients with suspected cirrhosis, as it is non-invasive and widely available. Among ultrasonographic signs of cirrhosis, liver surface nodularity, evaluated by high-resolution ultrasound at the left liver lobe (LLS), is the most accurate [11–14]. As the main limitation of the technique is its operator dependency [11], it is possible that an objective method to measure LLS may increase its accuracy and applicability.

The aims of this study were (1) to investigate the performance of LLS in the diagnosis of cirrhosis in patients with clinical suspicion of cirrhosis but also a differential diagnosis for the presenting abnormalities, who were chosen over those with an established diagnosis of cirrhosis to mirror the clinical situation in which further invasive investigation would be pursued; (2)

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to assess whether a digital analysis of images of LLS permits a valid, objective and quantitative LLS measurement; (3) to test the accuracy of TE for the diagnosis of cirrhosis in patients with clinical suspicion of this condition; and (4) to test the hypothesis that the combination of TE and LLS is more accurate than one single method for the non-invasive diagnosis of cirrhosis.

Materials and methods

Patients

This prospective study included all consecutive patients with a clinical suspicion of cirrhosis due either to long-lasting chronic liver disease or to clinical or laboratory symptoms in the presence of confounding comorbidity, consecutively referred for HVPG measurement ± transjugular liver biopsy (TJLB) to the Hepatic Haemodynamics Laboratory of the Hospital Clinic between May 2007 and August 2008. Suspicion of cirrhosis was raised by expert hepatologists of a teaching hospital of Barcelona (Hospital Clinic) and was based on the finding of physical or laboratory signs indicating liver failure and/or portal hypertension (Table 1).

Patients with an established diagnosis of cirrhosis were excluded from this study. In all patients the differential diagnosis included other conditions, most commonly non-cirrhotic portal hypertension and haematological malignancy (Table 1).

In the study period 96 eligible patients were observed; in 6, TE could not be performed due to temporary unavailability of the equipment due to periodic maintenance; these patients were excluded from the study. Therefore, the final cohort included 90 patients with suspected cirrhosis (61 males, 29 females; 54 ± 15 years). The main clinical, laboratory and haemodynamic characteristics of the studied population are summarised in Table 2.

Twelve healthy subjects (seven males, five females, age 38 ± 10 years) with normal liver tests were used as controls for non-invasive measurement of LLS.

Patient histories and routine laboratory tests were obtained at inclusion. The severity of liver disease was assessed by Child–Pugh [15] and MELD scores [16]. The study protocol was approved by the Ethics Committee of the Hospital Clinic. The nature of the study was explained to the patients, and informed consent was obtained in each case, according to the principles of the Declaration of Helsinki (revision of Edinburgh 2000).

High-resolution ultrasound of the liver lobe surface (LLS) and its quantification (qLLS)

Liver surface was studied at the left liver lobe by a portable Sonosite 180 Plus ultrasound equipment (SonoSite, Inc., Bothell, WA, USA) using a L38/10–5 MHz broadband linear array transducer. Examination was performed on the same day of hepatic vein catheterisation. To permit blind evaluation, LLS was studied before the invasive procedure in all cases. LLS was evaluated by a single experienced operator (AB), and images were blindly re-evaluated by another operator without specific experience in ultrasonography (JB). US examination lasted less than 2 min in all cases. During examination LLS was scored as smooth, irregular, or nodular. As per previously published data [11,13], LLS was considered to

Table 1. Main characteristics of the studied population (*n* = 90). *14 additional patients had concomitant ascites on ultrasound examination (none of them had clinically apparent ascites). 5 were referred for worsening of liver function, all of them in the context of possible alcoholic hepatitis; 5 were referred for esophageal varices; 2 were referred for variceal haemorrhage and 2 were referred for jaundice. LD = liver disease. SLE = Systemic Lupus Erythematosus.

Main indication for HVPG ± transjugular liver biopsy	Comorbidity	Main differential diagnosis	Cirrhosis at final diagnosis
Worsening of liver function in patients with long-lasting chronic LD (n = 42)			
HCV- or HBV-related chronic LD (n = 21)	Liver failure (n = 2); potentially resectable hepatocarcinoma (n = 6); inconclusive imaging/biochemistry (n = 13)	Cirrhosis; chronic hepatitis without cirrhosis and without portal hypertension; haematological malignancy (n = 4)	N = 20 (48%)
Chronic alcoholic LD (n = 13)	Acute alcoholic hepatitis (n = 8)		
Post-OLT recurrent HCV (n = 6)	HIV (n = 1); imaging compatible with chronic LD (n = 5)		
Primary biliary cirrhosis (n = 1)	Sigma neoplasia		
NASH (n = 1)	Obesity		
Clinical signs suggesting cirrhosis with compatible laboratory tests (n = 25)			
Clinical evidence of ascites (n = 12)*	Cardiac disease and alcohol consumption or HCV infection (n = 3); strong alcohol consumption but absence of signs of cirrhosis at imaging (n = 3); unknown cause and normal liver imaging (n = 3); post-OLT (n = 1); colon neoplasia (n = 1); HIV and cured HCV infection (n = 1)	Cirrhosis; congestive liver; alcoholic hepatitis; neoplastic liver; non-cirrhotic portal hypertension	N = 16 (64%)
Variceal hemorrhage (n = 7)	HIV (n = 2); HCV + mieloproliferative syndrome (n = 2); Crohn's disease treated with methotrexate (n = 1); cryptogenic LD and splenic thrombosis (n = 1); long-term abstinent alcoholic LD patient (n = 1)	Cirrhosis; non-cirrhotic portal hypertension; haematological disease	
Jaundice without biliary tree dilatation (n = 6)	Alcohol consumption (n = 3); suspected pancreas neoplasia in HCV infection (n = 1); HBV infection (n = 1); cerebral lymphoma (n = 1)	Cirrhosis;toxic hepatitis; haematological disease with liver infiltration	
Endoscopic or US signs of portal hypertension (n = 21)			
Esophageal or gastric varices (n = 10)	HIV infection and imaging suggesting chronic LD (n = 3); absence of detectable causes (n = 3); HBV infection and normal liver function (n = 2); diabetes, art. hypertension and normal liver function (n = 1); SLE (n = 1)	Cirrhosis; non-cirrhotic portal hypertension	N = 7 (33%)
Esophageal varices and portal or mesenteric vein thrombosis (n = 6)	HIV infection (n = 3); myeloproliferative disorder (n = 1); lymphoproliferative disorder and alcohol consumption (n = 2)	Cirrhosis; non-cirrhotic portal hypertension; haematological disease	
Splenomegaly and enlarged portal vein (n = 5)	HIV infection with history of cured HCV infection (n = 2); absence of detectable causes (n = 2); monoclonal gammopathy (n = 1)	Cirrhosis; non-cirrhotic portal hypertension; haematological disease	
Biochemical signs suggesting cirrhosis (n = 2)			
Increased INR, thrombocytopenia and elevated AST (n = 2)	Obesity (n = 1); absence of detectable causes (n = 1)	Cirrhosis; NASH without cirrhosis; other LD	N = 1 (50%)

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Table 2. Main characteristics of the patients included in the study. Data are shown as mean ± SD for normally distributed variables and as median (range) for non-normal variables. *p* Values refer to the comparison between patients with and without cirrhosis according to the gold standard.

	Overall	Cirrhosis	No cirrhosis	*p*
	(n = 90)	(n = 44)	(n = 46)	
Age (yrs)	53.7 ± 15.44	56.7 ± 11.9	50.8 ± 17.9	0.068
Gender (M/F)	61/29	28/16	33/13	0.411
Bilirubin (mg/dl)	1.25 (0.1–30.4)	1.4 (0.3–30.4)	1.1 (0.1–29.0)	0.087
INR	1.32 ± 0.35	1.39 ± 0.40	1.25 ± 0.30	0.063
Albumin (g/dl)	35 ± 8	32 ± 8	37 ± 8	0.005
Hemoglobin (g/dl)	12.4 ± 2.4	11.9 ± 2.4	12.8 ± 2.3	0.104
Leukocytes (n^3/mmc)	6.0 ± 3.0	6.2 ± 3.5	5.8 ± 2.4	0.476
Platelets (n^3/mmc)	138 ± 80	125 ± 62	150 ± 94	0.164
Creatinine (mg/dl)	0.89 (0.40–6.37)	0.84 (0.40–6.37)	0.92 (0.52–6.10)	0.260
AST (U/l)	61 (12–1775)	75 (12–602)	44 (17–1775)	0.219
ALT (U/l)	41 (10–2219)	39 (10–337)	42 (13–2219)	0.880
AST/ALT ratio	1.13 (0.32–5.82)	1.23 (0.52–2.67)	0.97 (0.32–5.82)	0.005
GGT (U/l)	101 (18–1223)	101 (18–1841)	123 (7–1233)	0.930
FAL (U/l)	217 (67–1917)	206 (67–532)	239 (98–1917)	0.294
APRI	0.51 (0.04–12.7)	0.61 (0.12–4.73)	0.35 (0.04–12.7)	0.173
Varices (%)*	54%	55%	53%	0.889
Ascites (%)	29%	43%	15%	0.003
TE (kPa)	24.9 ± 21.9	40.6 ± 22.6	11.8 ± 8.6	<0.0001
Nodular LLS (%)	39%	73%	7%	<0.0001
HVPG (mm Hg)	10.7 ± 6.6	15.0 ± 6.0	6.4 ± 3.7	<0.0001
HVPG ⩾ 210 mm Hg (%)**	53%	86%	25%	<0.0001

* These figures refer to a subgroup of 48 patients in whom gastroesophageal varices had been screened on inclusion as a part of the diagnostic work-up. In 10 of these patients the finding of varices was the principal indication for histological and hemodynamic evaluation (Table 1).
** These figures refer to patients in whom liver biopsy was performed and valid (n = 75).

exclude cirrhosis if smooth, indeterminate if irregular, and diagnostic of cirrhosis if nodular.

Three representative images were recorded. For the objective evaluation of LLS (quantitative LLS, qLLS), images were analysed SiteLink Image Manager version 3.4.1 (Sonosite Inc, Bothell, WA, USA) and were successively post-processed with digital image analysis software (PhotoLine version 14.51, Computerinsel, Bad Gögging, Germany). The liver surface was highlighted and semi-automatically measured in a standardised linear segment of 2 cm (Fig. 1). The software automatically produces a length of the segment of liver surface, as seen in Fig. 1. This length is expressed in centimetres with two decimals. On post-processing, the operator was blinded to the previous score assigned to LLS. Intraobserver and interobserver reproducibility of qLLS measurements were evaluated in 10 patients extracted randomly from the study population of the study independent operators blinded to the final diagnosis.

Transient elastography

TE was evaluated by Fibroscan® (Echosens, Paris, France). Measurements of liver stiffness were performed on the right lobe of the liver through intercostal spaces on patients lying in the dorsal decubitus position with the right arm in maximal abduction. The tip of the probe transducer was placed on the skin between the ribs at the level of the right hepatic lobe. The operator, assisted by an ultrasonic time-motion image, located a liver portion of at least 6 cm thick free of large vascular structures. Ten successful measurements were performed on each patient. Success rate was calculated as the ratio of the number of successful measurements to the total number of acquisitions. Only liver stiffness measurements with a success rate of at least 60% and an interquartile range lower than 30% were considered reliable. The results are expressed in kilopascals (kPa), and median values are representative of liver stiffness. The whole examination lasted less than 5 min.

Following previous findings [17], TE was considered to exclude cirrhosis at <12 kPa, indeterminate at 12–18 kPa, and to diagnose cirrhosis if ⩾18 kPa. A separate analysis was performed using a single cut-off of 13 kPa, as suggested by a recent meta-analysis [18].

Combination of LLS and TE

To evaluate the accuracy of the combination of the two non-invasive methods, those patients with an indeterminate result by one method and a positive result in the other were diagnosed cirrhotic; patients with two indeterminate results

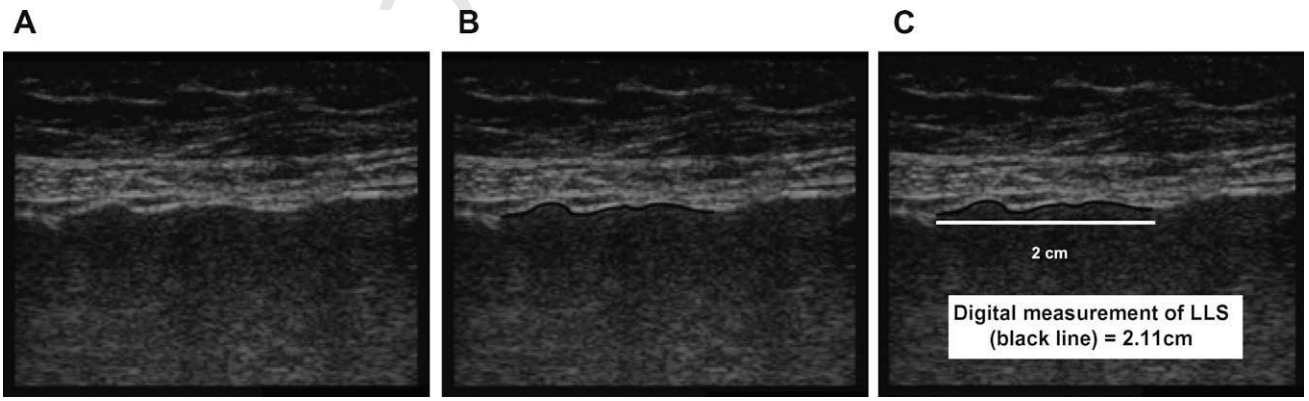

Fig. 1. Measurement of a segment of the left hepatic lobe surface (LLS). A representative image of LLS was used (A); with image analysis software, a linear segment of 2 cm was outlined (B and C). The software permitted the automatic measurement of the curve line identifying the liver surface matching the 2-cm linear segment (C). In the case shown below, a nodular LLS was observed, measuring 2.11 cm. Cirrhosis was confirmed by histology.

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were classified as indeterminate, as were those with discordant results (i.e., TE positive and LLS negative or LLS positive and TE negative). Patients with an indeterminate result in one of the two and a negative result in the other were diagnosed as not having cirrhosis.

HVPG measurement and TJLB

Immediately after ultrasound examination, patients underwent hepatic vein catheterisation. Under local anaesthesia, an 8F venous catheter introducer (Axcess; Maxxim Medical, Athens, TX, USA) was placed in the right internal jugular vein using the Seldinger technique. Thereafter, a 7F balloon-tipped catheter (OB-Medi-Tech, Boston Scientific Cork Ltd., Cork, Ireland) was advanced into the right hepatic vein to measure wedged and free hepatic venous pressures (WHVP and FHVP, respectively) by connection to an external electro-mechanical transducer and polygraph (Marquette Electronics, NY, USA). Hepatic venous pressure gradient was calculated as the difference between wedged and free hepatic venous pressure, as previously described [19]. All measurements were performed in triplicate, and permanent tracings were recorded. After HVPG measurement, when indicated, TJLB was performed under X-ray videofluoroscopy either by the aspiration technique (15-G needle; Cook Europe, Denmark) or, in the case of a small or fragmented specimen, by a Tru-Cut needle (18-G Tru-Cut needle; Cook Europe, Denmark). Passes of the needle were repeated until a satisfactory sample (total length at least 15 mm) was obtained [20]. Specimens were evaluated by a single expert pathologist at our Hospital, who is skilled in the interpretation of transjugular liver samples.

Diagnosis of cirrhosis was obtained on the base of histological examination or, in its absence, by the finding of clinically significant sinusoidal portal hypertension (HVPG $\geqslant$ 10 mm Hg) and compatible clinical and laboratory data. The diagnosis of idiopathic portal hypertension was suggested by the pathologists the after discussing the cases with the clinicians (integration of clinical, biochemical and histological data).

Statistical analysis

Following current recommendations for studies on diagnostic methods [21], we performed a phase I and II diagnostic study on qLLS to confirm that it was different between healthy subjects and cirrhotic patients. The comparison between these two groups was assessed by unpaired Student's *t*-test, and a cut-off for LLS quantification suggesting cirrhosis was obtained by analysing the receiver operating characteristics (ROC) curve. Phase III questions on the ability of TE and LLS to distinguish patients with and without cirrhosis in a situation of clinical suspicion were addressed through the acceptance of independent and blind gold standard (TJLB and HVPG); moreover, we used previously published cut-offs for TE and LLS to diagnose cirrhosis by non-invasive means.

Multilevel likelihood ratios (LRs) were used to explore the association of the presence of cirrhosis with liver stiffness and LLS. Ninety-percent confidence intervals [22] were calculated with CIA® statistical software (Version 2.1.2; University of Southampton, Southampton, UK). LRs above 10 and below 0.1 were considered to provide strong evidence to rule in or rule out diagnosis, respectively [23]. Accuracy analysis was performed with an intention-to-treat method, and, accordingly, technical failures of the non-invasive methods were considered 'indeterminate'.

Concordance between TE and LLS was evaluated by the unweighted kappa method with a specifically designed SPSS macro (Bonillo, Granero & Domenech, Universitat Autonoma de Barcelona, 2003). The concordance coefficient (k) was graded by the scale proposed by Landis and Koch [24]. Intra- and interobserver variability of qLLS measurements were evaluated by the intraclass correlation coefficient (ICC).

The normality of distribution of continuous variables was tested by the Kolmogorov–Smirnov test. Comparisons between groups for numerical continuous variables were made by an unpaired Student's *t*-test (normally distributed variables) or Mann–Whitney *U* test (non-normally distributed variables) and for frequencies by Fisher's test. The α value was set at 0.05. All p values were two-sided. Statistical analysis was performed with SPSS 16.0 package (SPSS, Chicago, IL, USA).

Results

Diagnosis of cirrhosis by gold-standard techniques

Overall, cirrhosis was diagnosed in 44 patients (32 by means of histology) and ruled out in 46.

Liver biopsy was performed in 76/90 (84%) patients; in one case it was not diagnostic due to an insufficient specimen. In 32 of these patients the final diagnosis was cirrhosis, while the 43 non-cirrhotic patients were diagnosed with normal or near-normal liver (n = 9), HCV-related chronic hepatitis with F2–F3 fibrosis (n = 4), idiopathic portal hypertension (n = 4), unspecific chronic hepatitis with no or minimal fibrosis (n = 4), regenerative nodular hyperplasia (n = 4), simple steatosis (n = 3), alcoholic hepatitis with sinusoidal fibrosis (n = 1), non alcoholic steatohepatitis (n = 2), acute toxic hepatitis (n = 2), acute alcoholic hepatitis (n = 2), T cell lymphoma (n = 2), cardiac congestive liver (n = 3), alpha-1 antitrypsin chronic liver disease (n = 1), infiltration from solid neoplasia (n = 1) and plasma cell infiltration (n = 1).

In patients without or with insufficient liver samples, cirrhosis was finally diagnosed on the basis of the presence of clinically significant portal hypertension (CSPH), physical/laboratory data and imaging techniques.

HVPG measurement was successful in 89/90 patients; in one patient the presence of veno-venous communications prevented HVPG measurement. This patient was diagnosed with idiopathic portal hypertension by TJLB and compatible clinical data.

LLS and qLLS

LLS could be studied in 89/90 patients; in one case obesity prevented LLS examination (Fig. 2). Agreement in the assessment between two observers, an expert ultrasonographist (AB) and a hepatologist with minimal teaching but without specific training in ultrasound (JB), was tested in 15 randomly chosen patients and was excellent (k = 0.9).

On semi-quantitative evaluation, LLS was scored as smooth in 23 patients, irregular in 31 and nodular in 35. Control subjects showed smooth LLS in all cases.

On an intention-to-treat analysis, LLS diagnosed cirrhosis in 35 patients (39%) and ruled out cirrhosis in 23 (26%). In the remaining 32 patients (35%), LLS was indeterminate. Therefore, the finding of nodular LLS had a +LR of 11.15 (90% CI 4.38–28.36) for ruling in cirrhosis, while the finding of smooth LLS had a −LR of 0.10 (90% CI 0.03–0.32) for ruling out cirrhosis (Table 3).

On post-processing of images, qLLS was significantly different in the three categories (ANOVA $p < 0.0001$): 2.00 ± 0.01 in smooth liver surface, 2.04 ± 0.04 in irregular liver surface ($p = 0.001$ vs. smooth) and 2.09 ± 0.04 in nodular surface ($p < 0.0001$ vs. irregular and vs. smooth). Similarly, qLLS was 2.00 ± 0.01 cm (range 1.99–2.01 cm; median 2.00 cm) in healthy subjects and 2.09 ± 0.03 cm (range 2.02–2.22 cm; median 2.09 cm) in patients with proven cirrhosis ($p < 0.0001$). In the study cohort (i.e., excluding controls) qLLS showed an AUROC of 0.88 (90% CI 0.81–0.96) ($p < 0.0001$) for the diagnosis of cirrhosis. The best cut-off for identification of cirrhosis was 2.04, which had a sensitivity of 89% (90% CI 0.78–0.94), specificity of 82% (90% CI 0.71–0.90), +LR of 6.01 (90% CI 3.01–9.67) and −LR of 0.14 (90% CI 0.07–0.28).

The analysis of the subgroup of patients with irregular liver surface, who were indeterminate at semi-quantitative evaluation, showed that a qLLS < 2.04 decreased the probability of having cirrhosis from 35% to 18%, and the observation of a value $\geqslant$ 2.04 increased this probability from 35% to 57%.

Intraobserver and interobserver reproducibility of qLLS were assessed in 10 patients randomly chosen from the studied popu-

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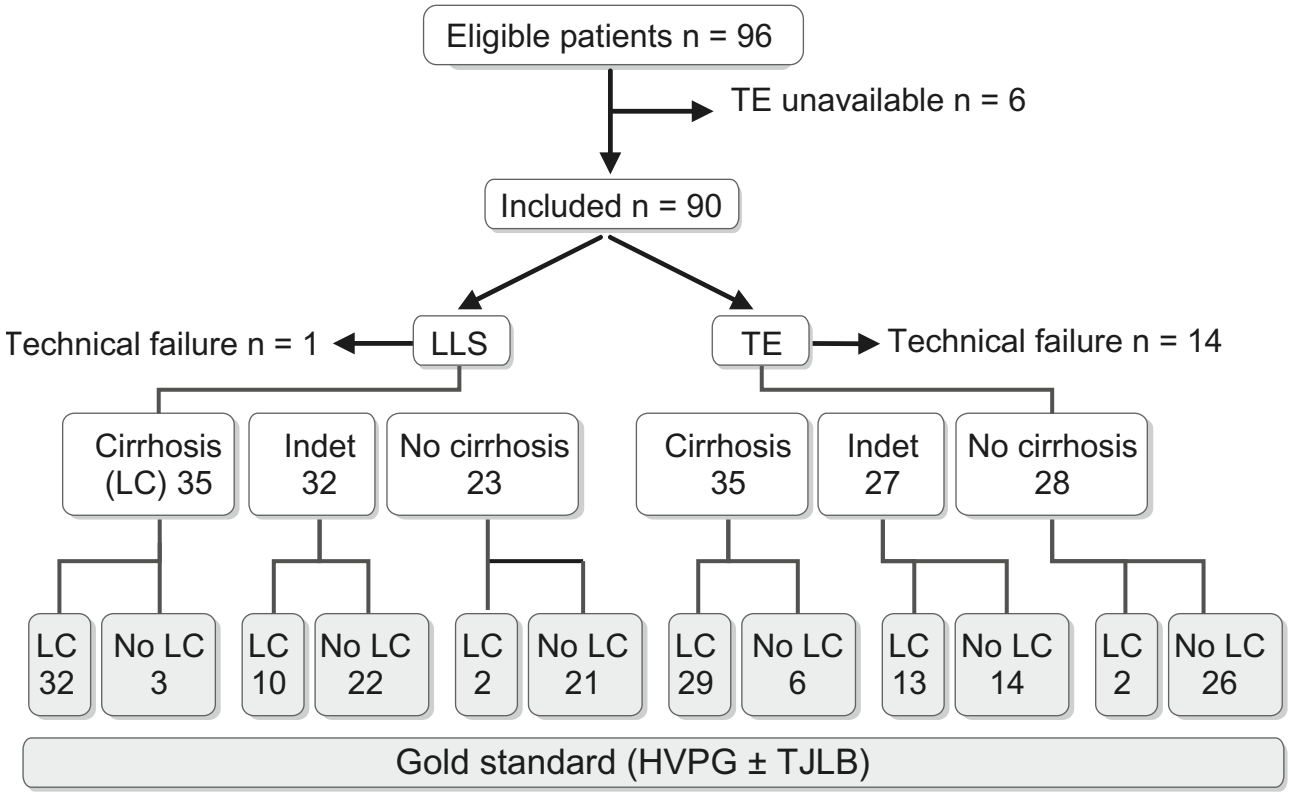

Fig. 2. Algorithm of the study and diagnosis according to non-invasive methods and the gold standard in the entire study population ($n = 90$).

lation by two independent observers, both blinded to the final diagnosis results. Both performed three measurements on the images of the 10 subjects. Intraobserver reproducibility was near optimal: observer 1 showed an intraclass correlation coefficient (ICC) of 0.969 (95% CI 0.913–0.991) for absolute concordance. Observer 2 had an ICC of 0.954 (95% CI 0.875–0.987) for absolute concordance. The method was also highly reproducible between the two observers, as confirmed by an ICC of 0.915 (95% CI 0.682–0.979) for absolute concordance.

Transient elastography (Fig. 2)

TE could be correctly performed in 76/90 patients. In 13, the measurement was not feasible due to obesity ($n = 5$), ascites ($n = 5$), lack of cooperation by the patient ($n = 2$) and small right hepatic lobe ($n = 1$). In one case the measurement was performed but not valid due to a low success rate (44%). The rate of technical failures was significantly greater for TE as compared to LLS (15.5% vs. 1.1%, $p = 0.001$). In patients in whom TE could be measured, it showed a near-optimal accuracy for the diagnosis of cirrhosis (AUROC 0.91; 90% CI 0.84–0.97; $p < 0.0001$).

According to the previously mentioned cut-offs (<12 kPa ruled out cirrhosis, 12–18 kPa was indeterminate, >18 kPa diagnosed cirrhosis), in an intention-to-treat analysis, TE diagnosed cirrhosis in 35 patients (39%) and ruled out cirrhosis in 28 (31%) (Table 3). In the remaining patients (30%) TE was indeterminate. According to these data, +LR was 5.05 (2.63–9.71) and −LR 0.08 (0.03–0.26).

Even including in the analysis only patients in whom TE was measurable (per-protocol analysis), the performance of TE improved only slightly. A value over 18 kPa had a +LR of 5.97 (3.17–11.26), while a TE < 12 kPa had a −LR of 0.10 (0.03–0.30), and 17% of patients in this analysis were indeterminate.

Table 3. Diagnostic performance of the two tested non-invasive techniques and their combination in the population of the study (n = 90).

Cirrhosis		% patients	Likelihood Ratio (LR) (90% CI)
TE	LC (⩾18 kPa)	39	5.05 (2.63–9.71)
	Non-determined/technical failure (12–18 kPa)	30	0.97 (0.57–1.65)
	No LC (<12 kPa)	31	0.08 (0.03–0.26)
LLS	LC (nodular)	39	11.15 (4.38–28.36)
	Non-determined/technical failure (irregular)	35	0.48 (0.28–0.80)
	No LC (smooth)	26	0.10 (0.03–0.32)
TE + LLS	LC	43	9.15 (4.12–20.32)
	Non-determined/technical failure	18	0.81 (0.38–1.73)
	No LC	39	0.06 (0.02–0.20)

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By using the 13 kPa cut-off, TE had a poor accuracy for diagnosing cirrhosis and a moderate accuracy for ruling out cirrhosis both in the intention-to-treat analysis [sensitivity 68% (90% CI 56–78%), specificity 59% (90% CI 47–70%),+LR 2.09 (90% CI 1.42–3.08) and −LR 0.15 (90% CI 0.07–0.35)] and in the per-protocol analysis [sensitivity 88% (90% CI 76–95%), specificity 64% (90% CI 52–75%), +LR 2.47 (90% CI 1.73–3.53) and −LR 0.18 (90% CI 0.08–0.41)].

Comparison of LLS and TE and their combination for the diagnosis of cirrhosis

TE correlated with qLLS ($R = 0.62$, $p < 0.0001$). The concordance between TE and semi-quantitative and quantitative evaluation of LLS for the diagnosis of cirrhosis was slight ($k = 0.27$, 95% CI 0.05–0.46, $p = 0.015$), while the concordance between the two methods for ruling out cirrhosis was moderate ($k = 0.40$, 95% CI 0.18–0.59, $p < 0.0001$). As shown in Table 3, LLS had the highest accuracy for ruling in cirrhosis (+LR: 11.15; 90% CI 4.38–28.36), while TE was the best non-invasive technique to rule out cirrhosis (−LR 0.08; 90% CI 0.03–0.26). The combination of the two methods markedly reduced the number of indeterminate patients (TE 30%, LLS 35%, combination 18%; Table 3) and maintained a good accuracy.

Results in patients diagnosed by liver biopsy

The performance of the two non-invasive methods in the 75 patients with histological diagnosis (cirrhosis $n = 32$) was similar to that observed in the whole study population (Fig. 3).

TE was technically feasible in 63/75 patients (84%). In these patients TE had an AUROC of 0.887 (90% CI 0.807–0.967; $p < 0.0001$) for the diagnosis of cirrhosis. In an intention-to-treat analysis, TE over 18 kPa had a +LR of 4.93 (90% CI 2.56–9.49), while TE < 12 kPa had a −LR of 0.08 (90% CI 0.02–0.41); 33% of patients were indeterminate.

In a per-protocol analysis, TE performed similarly; TE > 18 kPa had a +LR of 5.22 (90% CI 2.77–9.82), while TE < 12 kPa had a −LR of 0.07 (90% CI 0.01–0.35) for the diagnosis of cirrhosis.

By using the 13 kPa cut-off, TE had the following performance. Intention-to-treat analysis: sensitivity 72% (90% CI 57–83%), specificity 51% (90% CI 39–63%), +LR 2.06 (90% CI 1.90–3.04) and −LR 0.18 (90% CI 0.07–0.47); per-protocol analysis: sensitivity 88% (90% CI 74–95%), specificity 58% (90% CI 45–71%), +LR 2.12 (90% CI 1.5–3.0) and −LR 0.20 (90% CI 0.08–0.50).

LLS could be evaluated in all cases. The finding of nodular LLS had a +LR of 8.06 (90% CI 3.61–18.01) for ruling in cirrhosis, while the finding of smooth LLS had a −LR of 0.06 (90% CI 0.01–0.33) for ruling out cirrhosis, and 34% of patients were indeterminate. qLLS was measured in all patients and had an AUROC of 0.851 (90% CI 0.758–0.945; $p < 0.0001$) for the diagnosis of cirrhosis.

The combination of LLS and TE in these patients maintained good accuracy: +LR 8.74 (90% CI 3.96–19.31), −LR 0.09 (90% CI 0.03–0.29); after combination of the two non-invasive techniques, 15% of patients were indeterminate.

Discussion

The main result of the present study is that a simple non-invasive technique based on the ultrasonographic examination of the LLS had very good diagnostic accuracy to detect cirrhosis, similar to that of TE measurements by Fibroscan, a technique that has received much attention in recent years. In order to minimise the observer-dependency of LLS assessment, we applied a novel method based on the computerised post-processing of the ultrasound images, which permits quantification of LLS length in a standardised segment (qLLS); this method had high intra- and interobserver reproducibility in the present study, and it let us compare the accuracy of TE and LLS, which proved to be analogous for the diagnosis of cirrhosis in an intention-to-treat analysis. However, the concordance between the two non-invasive

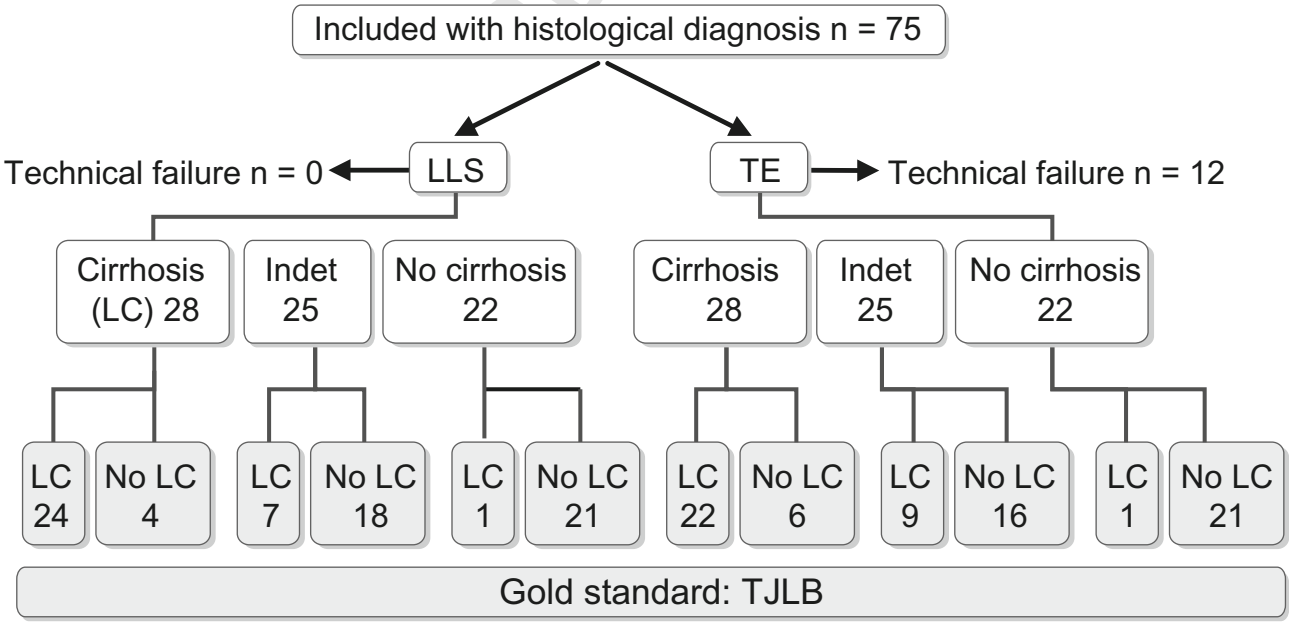

Fig. 3. Diagnosis according to non-invasive methods and the gold standard in the subgroup of patients diagnosed by biopsy ($n = 75$).

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techniques was only moderate. TE and LLS are applied to different lobes, and differences in the degree of fibrosis might explain part of the variability. Still, the lack of concordance most likely reflects the fact that TE and LLS evaluate different unique characteristics of cirrhosis: TE senses increased liver stiffness, mainly due to fibrosis, while LLS visualises the nodules. Because of this, it is not surprising that their combination is superior to either one alone [25]. In a sense, the combination of these two techniques integrates, in a quantitative and objective way, the information that skilled physicians have long recognised, that palpating a firm, rough liver edge strongly indicates cirrhosis, as do the visualisation of a nodular liver surface at surgery, peritoneoscopy, or CT/MR scans.

Of interest, the finding of nodular LLS was superior to high values of TE for ruling in cirrhosis, while low values of TE were slightly superior to smooth LLS to rule out this diagnosis. More important, the combination of the two methods markedly reduced the number of indeterminate cases, from 30% (TE) and 35% (LLS) to 18%.

This is relevant in the clinical diagnosis of individual patients in whom TE values are in the 'grey zone' or not feasible. In this regard, it is worth noting that in this series TE had a 15% rate of technical failure. This is higher than reported in previous studies [26] and may be partly due to the inclusion of patients with ascites in our population. LLS evaluation, on the contrary, could be obtained in all patients but one, including obese and ascitic patients in whom TE was not obtainable, suggesting that its applicability is very high and that LLS assessment may be helpful for the classification of patients in whom TE cannot be performed. Although it can be argued that the presence of ascites makes TE unnecessary because the diagnosis of cirrhosis may be obvious, this was not the case in the present series, which intentionally included patients with a suspicion of cirrhosis but in whom other diagnoses were also likely. Actually, ascites was not due to cirrhosis in one-third of our patients with ascites. It should be noted that the accuracy of TE improved only slightly, even including in the analysis only patients in whom TE was measurable.

In this study, the semi-quantitative assessment of LLS was superior to qLLS in diagnosing and excluding cirrhosis but gave a substantial number of indeterminate cases. Because of this limitation, it is important to note that qLLS, which provides a quantitative, operator-independent evaluation of LLS, ameliorated the performance of LLS in indeterminate cases.

As per STARD guidelines [27], we decided to apply previously published cut-offs for the diagnosis of cirrhosis by TE; we first used the cut-offs derived from a study conducted in patients with HCV-related chronic liver disease [17] because they offered the best methodological approach. Cut-offs for TE have been shown to vary according to the cause of the underlying liver disease [28,29], so it has been suggested that it may be better to choose TE cut-offs according to the aetiology [30]. However, in actuality the cause of chronic liver disease is frequently multifactorial or unknown at the time of initial examinations, and specific cut-offs are not available for all aetiologies. Therefore, we also applied a single cut-off of 13 kPa, which has been suggested as an 'optimal' cut-off in a recent meta-analysis [18]. TE performed poorly in our series using this cut-off.

On the other hand, it may be argued that, instead of assessing only LLS, as we chose, if we had performed a complete abdominal ultrasonographic study, this would have given more detailed information. Still, we wanted to identify and test a simple, user-friendly, rapid and operator-independent sign, which could be applicable at bedside similarly to TE by a non-specifically expert operator, and LLS and qLLS evaluation fulfilled all these requirements. In this regard it should be noted that even the subjective appreciation of smooth, irregular and nodular appearance of LLS exhibited excellent agreement between observers, and the portable ultrasonic equipment used is low-cost and readily available in many centres, as it is analogous to that commonly used at bedside for the US guidance of central venous access and other invasive procedures.

The importance of conducting clinical studies in patients who are typical of day-to-day clinical care has been recently emphasised [31]. To assess the actual usefulness of the two non-invasive methods in clinical practice, we included only patients with clinical suspicion of cirrhosis raised in highly specialised hospitals but with a strong differential diagnosis challenging the clinical assessment. As shown by our results, clinical uncertainty was justified, as half of the included patients did not have cirrhosis after full work-up, including TJLB and HVPG evaluation. Nonetheless, due to the peculiar clinical setting of our study, other studies are needed in order to confirm our results in other populations at risk of cirrhosis, such as asymptomatic patients with chronic liver diseases.

In conclusion, our data suggest that the diagnosis of cirrhosis can be achieved through non-invasive methods with high accuracy, even in difficult clinical circumstances. The combination of transient elastography and high-resolution ultrasonographic evaluation of the liver surface increases the ability of the two separate methods to diagnose and rule out cirrhosis.

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