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TRANSIENT ELASTOGRAPHY PREDICTS FIBROSIS PROGRESSION IN PATIENTS WITH RECURRENT HEPATITIS C AFTER LIVER TRANSPLANTATION

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Abbreviations
LB: Liver biopsy
TE: Transient elastography
LT: Liver transplantation
HCV: Hepatitis C virus
AIH: Autoimmune hepatitis
NASH: Nonalcoholic steatohepatitis
BMI: body mass index
AUROC: Area under receiver operating characteristics
UNL: Upper normal limit

G: necroinflammatory grading

S: fibrosis staging

ABSTRACT

Objective: Transient elastography (TE) allows non-invasive evaluation of liver disease severity in patients with chronic hepatitis C. This procedure, however, warrants further validation in the setting of liver transplantation (LT), including patients under follow-up for recurrent hepatitis C.

Setting: Tertiary referral hospital.

Patients: 95 patients (75 males) transplanted for end-stage liver disease due to hepatitis C virus.

Interventions: Paired liver biopsy (LB) and TE were carried out 6 to 156 (median 35) months after LT. 40 patients with recurrent hepatitis C sequentially evaluated 6 to 21 months apart.

Main outcome measures: Clinical, laboratory and graft histological features influencing TE results.

Results: Median TE values were 7.6 kPa in the 90 patients with a successful TE examination, being 5.6 kPa in the 30 patients with Ishak fibrosis score (S) of 0-1, 7.6 kPa in the 38 with S2-3; 16.7 kPa in the 22 with S4-6, ($p<0.0001$). The areas under ROC curves were 0.85 (95%CI: 0.76-0.92) for S≥3, 0.90 (95%CI: 0.82-0.95) for S≥4 with 7.9 and 11.9 kPa optimal TE cut-off (81% and 82% sensitivity, 88% and 94% negative predictive value, respectively). Fibrosis, necroinflammatory activity and higher than 200 IU/L gamma-glutamyl transpeptidase levels independently influenced TE results. During post-LT follow-up, TE results changed in parallel with grading ($r=0.63$) and staging ($r=0.71$), showing 86% sensitivity and 92% specificity in predicting staging increases.

Conclusions: TE accurately predicts fibrosis progression in LT patients with recurrent hepatitis C, suggesting that protocol LB might be avoided in patients with improved or stable TE values during follow-up.

Non-invasive assessment of hepatitis C progression has been advocated in an attempt to bypass the drawbacks of liver biopsy (LB),[1] mainly represented by high cost and a small risk of severe complications.[2] Virtually all serological tests developed to date have not entirely met clinicians' expectations, as they insufficiently predicted on an individual level the stage of liver fibrosis, which is the relevant determinant of prognosis in chronic liver diseases.[3,4] Recently, transient elastography (TE, Fibroscan®) gained popularity as a user-friendly non-invasive technique for measuring liver stiffness,[5] which has been shown to accurately predict liver fibrosis in a variety of clinical conditions including hepatitis C.[5-12] TE in patients with chronic hepatitis C has proved to be highly reproducible approaching 0.98 inter- and intra-observer agreement rates.[13] In liver transplanted patients, in whom histological evaluation of the graft is of paramount importance for their management, protocol LB remains the reference standard for assessing liver fibrosis progression and graft disease severity.[14] To date, non-invasive tests for liver fibrosis have not been fully validated in this setting. In transplanted patients, in fact, the diagnostic accuracy of serological tests is likely to be even more weakened than in the non-transplant setting by changes in serum biochemical markers that occur independently of the severity of liver disease. In addition, occurrence of multiple etiology graft damage apart from recurrent hepatitis C, like rejection and surgical complications,[15,16] may interfere with TE evaluation of the graft. The only study assessing TE in liver transplanted patients with recurrent hepatitis C, showed a good correlation of liver stiffness with histological scores of liver fibrosis and hepatic vein pressure gradient, but it was limited to a cross-sectional analysis of the patients.[17]

In the present study, we tried to identify both graft- and host-related features that might influence TE results in patients with recurrent hepatitis C and assessed the ability of TE to detect liver fibrosis progression during follow-up.

PATIENTS AND METHODS

Patients

Between September 2005 and September 2007 we investigated all patients transplanted for end-stage liver disease due to hepatitis C virus (HCV) infection who consecutively underwent either on demand or protocol LB to assess the graft status. LB was performed at least 6 months following liver transplantation (LT) concurrently with TE examination and blood chemistries. Exclusion criteria were chronic graft rejection, inadequate LB or unsuccessful TE examination. All patients were non-drinkers as assessed by a thorough interview. A written informed consent was obtained from all patients.

Diagnostic criteria

Recurrent hepatitis C was defined as the presence of histopathological features of acute or chronic hepatitis in serum HCV-RNA positive patients by PCR any time point following LT, independently of transaminase values.[18] De novo AIH was the presence of high transaminase levels (>x 1.5 UNL), ≥1:80 serum titres tissue autoantibodies, >1.5 g/dL immunoglobulin G and interface hepatitis with portal plasma cell infiltrates occurring in patients transplanted for non autoimmune liver disease.[19] Idiopathic, post-LT hepatitis was graft damage with features of chronic hepatitis of undefined etiology, without conspicuous bile duct damage and venous endothelial inflammation.[20] De novo nonalcoholic steatohepatitis (NASH) was the association of predominant macrovescicular steatosis, ballooning cell degeneration, Mallory's hyaline, perisinusoidal fibrosis, and neutrophilic lobular infiltration in non drinkers.[21]

Laboratory investigations

Serum alanine aminotransferase (ALT), aspartate aminostransferase (AST), γ-glutamyltranspeptidase (γ-GT) and alkaline phosphatase (ALP) activities were measured by an automatic method at 37° C (normal value ALT and AST ≤32 IU/L in women and ≤38 IU/L in men; γ-GT ≤40 IU/L; ALP ≤120 IU/L). Serum anti-HCV was detected by a second-generation assay (Ortho DS, Raritan, NJ). Serum HCV-RNA was assessed by RT-PCR assay. Anti-nuclear (ANA), anti-smooth muscle antigen (SMA), anti-mitochondrial (AMA) and anti-liver kidney microsomal (LKM-1) antibodies were searched for by an indirect immuno-fluorescence assay performed on 4 μm cryostat sections from rat liver, kidney and stomach at a sera dilution of 1:40. All sera with ANA reactivity were further characterized on Hep-2 cells (INOVA Diagnostics, Inc., San Diego) at 1:80 sera dilution. Positive sera were titred off at 1:320 dilution.
The AST to platelet ratio index (APRI) was calculated as the actual AST concentration divided by its upper normal limit / platelet count (10^9/L) × 100.[22]

Liver biopsy

All patients were offered a LB whenever it was felt necessary by the attending clinician (on demand LB) or according to an established protocol (protocol LB: at month 6 and annually thereafter). Patients with a platelet count above 50,000/μL and lower than 1.3 international normalized ratio (INR) were investigated with an

ultrasound-guided percutaneous transthoracic LB using an automated Menghini needle (16 gauge, Biomol, Hospital Service, Rome, Italy). Patients with a platelet count below 50,000/μL or higher than 1.3 INR were assigned to the transjugular liver biopsy. Liver tissue cores were considered adequate if longer than 15 mm or with ≥11 complete portal tracts. Formalin-fixed and paraffin-embedded 5 micron-thick sections of liver tissue were stained with hematoxylin-eosin and Masson trichrome. One experienced hepatopathologist (G.R.), blinded to clinical data and TE results, independently scored samples for necroinflammatory activity (grading 0-18) and fibrosis (staging 0-6) according to the Ishak score.[23] Steatosis was arbitrarily graded according to the number of fatty hepatocytes: none <5%; mild 5-24%; moderate 25-50%; severe >50%. Perisinusoidal fibrosis score was based on percent of zone 3 foci involved: 0 (0%); 1 (<33%); 2 (33-66%); 3 (>66%) as previously described for steatohepatitis.[21] Acute cellular rejection was graded according to the Banff criteria.[24] Overlapping features of late cellular rejection and HCV infection were recorded as previously described by Demetris.[25]

Transient elastography

The right lobe of the liver was scanned with TE (Fibroscan®, Echosens, Paris) through an intercostal space access, while the patient was lying in the dorsal decubitus position with the right arm in maximal abduction. Using the ultrasound guide of Fibroscan®, a liver portion of at least 6 cm thickness, free of large vessels, was identified to carry out TE examination. The rate of successful measurements was calculated as the ratio between the number of validated and total measurements. The examination was considered reliable if at least 10 validated measurements were obtained for each patient with a greater than 65% success rate and if interquartile range of all validated measurements was lower than 30% of the median value. The median value of successful measurements was considered as representative of liver stiffness, expressed in kilopascal (kPa).

Follow-up study

Forty patients sequentially underwent paired protocol LB and TE examinations. Changes in fibrosis staging and TE results were categorized as follows: increase/decrease of histological fibrosis was ≥1 point variation of staging; increase/decrease of TE was ≥30% variation of baseline value, the 30% threshold of TE changes corresponding to the accepted variability of TE assay.

Statistical analysis

Spearman correlation coefficients were used to evaluate the relationships between parameters. By the non-parametric tests Mann-Whitney U and Kruskal-Wallis we compared TE results among different fibrosis stages. The Fisher exact test was used to compare categorical variables. Optimal stiffness cut-off values were obtained by a receiver-operating characteristic (ROC) curve analysis. TE values distribution was normalized by log transformation. Univariate regression analysis was used to evaluate the influence of host-related features like age, gender and body mass index (BMI), biochemical tests like ALT, AST, γ-GT, ALP and total bilirubin, and histological features like fibrosis staging (S), necroinflammatory grading (G), perisinusoidal fibrosis and steatosis, on log transformed TE results. Multiple regression analysis was then performed to evaluate the simultaneous influence on log transformed TE

results of the variables that showed a statistically significant effect at univariate analysis. SAS software (9.1) was used for statistical analysis.

RESULTS

Patients

During the study period, 95 patients (75 males) who underwent LT between 1993 and 2007 were consecutively investigated with both a percutaneous transthoracic LB and TE. Five (5%) patients were excluded from the analysis because of chronic rejection (n=1) or unsuccessful TE examination (n=4), due to a biliary cyst in the right lobe (n=1), and overweight (n=3, BMI >31.5 Kg/m^2). None was excluded because of inadequate LB specimen. Table 1 describes the relevant demography and clinical features of the 90 patients included in the study.

Table 1. Demographic and clinical features of the 90 patients concurrently evaluated with both transient elastography and liver biopsy.

FEATURES	
Males, No.	73 (81%)
Age at transplantation, years *	54 (20−66)
Age at the time of the study, years *	58 (22−71)
Body mass index, Kg/m^2 *	24.8 (17.2−33.4)
HCV genotype 1, No.	68 (76%)
ALT and AST ≤1 UNL, No.	29 (32%)
γ-GT ≤1 UNL, No.	41 (46%)
Immunosuppressive therapy:	
Cyclosporin (CyA), No.	28 (31%)
Tacrolimus (FK506), No.	23 (26%)
CyA or FK506 plus mycophenolate mofetil, No.	37 (41%)
CyA or FK506 plus azathioprine, No.	2 (2%)

* Median (Range)

Paired LB and TE were carried out between month 6 and year 13 following LT (median: 35 months). HCV infection recurred in 89 (99%) patients transplanted for HCV-related end-stage liver disease. The 40 patients with recurrent hepatitis C had paired LB and TE sequentially performed 6 to 21 months apart. Table 2 shows the indications to LB and the etiology of graft disease in the 90 patients.

Table 2. Indication to liver biopsy (LB) and liver graft disease in the 90 patients studied.

		LIVER BIOPSY	
ETIOLOGY OF GRAFT DISEASE	PATIENTS	PROTOCOL (No.=80)	ON DEMAND (No.=10)
Recurrent hepatitis, No.	74 (82%)	72	2
Recurrent hepatitis + rejection, No.	11 (12%)	6	5
Recurrent hepatitis + steatohepatitis, No.	4 (5%)	1	3

Cholangitis, No.	1 (1%)	1	0

Laboratory investigations

Overall, median serum ALT, AST, γ-GT were 46 UI/L (range 11−384), 44 UI/L (range 17−391) and 55 UI/L (range 9−1232). Median APRI was 0.8 ranging from 0.2 to 8.6. APRI was not able to predict liver fibrosis stage (AUROC 0.59 for S≥2 and S≥3, 0.56 for S≥4, p=0.17 and p=0.42, respectively).

Liver biopsy

The length of liver tissue cores ranged from 16 to 60 mm (median: 32 mm). Eight specimens (8%) were between 16 and 20 mm, each containing however at least 11 portal tracts. All the 89 HCV-RNA positive patients had histological features of recurrent hepatitis C with degrees of disease severity ranging from minimal changes (G≤3 and S≤1, n=6) to extensive bridging fibrosis or incomplete/complete cirrhosis (S≥4, n=22). Eleven patients (14%) had features of late rejection overlapping with recurrent hepatitis. The HCV-RNA negative patient (1%) showed features of cholangitis due to biliary stricture. The TE results stratified according to liver fibrosis staging and necroinflammatory grading are shown in Table 3.

Table 3. Transient elastography results according to liver fibrosis stage (S) and necroinflammatory activity (G) in the 90 patients with recurrent hepatitis C.

HISTOLOGY	PATIENTS No.	TRANSIENT ELASTOGRAPHY kPa *
FIBROSIS STAGE		
S0-1	30 (33%)	5.6 (4.0−14.5)
S2-3	38 (42%)	7.6 (3.5−12.0)
S4	7 (8%)	12.0 (5.2−17.5)
S5-6	15 (17%)	20.2 (8.2−48.8)
NECROINFLAMMATORY ACTIVITY		
G0-3	12 (13%)	5.5 (4.2−13.0)
G4-8	57 (64%)	7.3 (3.5−34.3)
G9-12	21 (23%)	10.1 (3.9−48.8)
G13-18	0	

* Results are expressed as median (Range)

Transient elastography

The overall applicability of the test was 95%, i.e. 90 patients were successfully examined out of the 95 being studied, and the median success rate of the examinations was 100%, with a range from 67% to 100%. TE success rate was ≥80% in the 71 patients with a median BMI of 24.2 Kg/m^2 and <80% in the 19 with a median BMI of 26.4 Kg/m^2 (p=0.002). Overall, the median TE was 7.6 kPa, the 83 patients with G≥4 and S≥2 recurrent hepatitis showing higher TE values than the 7 patients with G≤3 and S≤1 (7.8 vs 5.5 kPa, p=0.002). None of the six patients with minimal changes (G≤3 and S≤1) had higher than 6.9 kPa TE values. Among the 11

patients with overlapping features of recurrent hepatitis C and late rejection, 9 S1-2 patients had TE results ranging from 6.1 to 14.5 kPa with a median value of 8.4 kPa, compared to the 6.2 kPa median TE in the 44 S1-2 patients lacking features of late rejection (p=0.007).

TE values significantly correlated with the histological scores for fibrosis (r=0.67, p<0.0001), being significantly different for each fibrosis stage (p<0.0001) (Figure 1).

TE values significantly correlated also with histological grading (r=0.43, p<0.0001), perisinusoidal fibrosis (r=0.24, p=0.03), serum ALT levels (r=0.26, p=0.01), AST levels (r=0.39, p<0.0001) and γ-GT levels (r=0.36, p<0.0001), but not with steatosis and BMI. In addition, TE values significantly correlated with histological grading in S1 patients (r=0.40, p=0.04), S4 patients (r=0.78, p=0.04), S5 patients (r=0.79, p=0.01) and with perisinusoidal fibrosis in S5 patients (r=0.90, p=0.006).

By univariate regression analysis staging (p<0.0001), grading (p=0.0001), perisinusoidal fibrosis (p=0.02) and higher than 200 IU/L γ-GT serum levels (p=0.007) were the variables significantly associated with log transformed TE results. By multiple regression analysis, staging (p<0.0001), grading (p=0.0001) and higher than 200 IU/L γ-GT serum levels (p=0.004) were the only independent influencers of TE results (Table 4).

Table 4. Features that significantly influenced transient elastography results in the 90 patients with recurrent hepatitis C.

FEATURES	UNIVARIATE REGRESSION		MULTIPLE REGRESSION *
Staging	p < 0.0001	(R^2=0.57)	p < 0.0001
Grading	p = 0.0001	(R^2=0.19)	p = 0.0001
Perisinusoidal fibrosis	p = 0.02	(R^2=0.06)	NS
γ-GT ≥ 200 IU/L	p = 0.007	(R^2=0.08)	p = 0.004

* The R^2 of the final model was 0.71.

By ROC curve analysis, three TE threshold values for liver fibrosis were identified, i.e. 6.3 kPa for S≥2 (83% sensitivity and 70% specificity), 7.9 kPa for S≥3 (81% sensitivity and 76% specificity) and 11.9 kPa for S≥4 (82% sensitivity and 96% specificity) (Table 5). The corresponding area under ROC curves (AUROC) were 0.78 (95% CI 0.68-0.86) for S≥2, 0.85 (95% CI: 0.76-0.92) for S≥3, and 0.90 (95% CI: 0.82-0.95) for S≥4 (Figure 2). Using the 11.9 kPa cut off value, TE showed similar sensitivity and specificity (93% and 91%, respectively) for the diagnosis of S≥5 (Table 5).

Table 5. Operative characteristics of transient elastography in assessing liver fibrosis stage.

STAGING	CUT-OFF	SENS	SPEC	+LR	-LR	PPV	NPV
≥ 2	6.3 kPa	83%	70%	2.8	0.2	85%	68%
≥ 3	7.9 kPa	81%	76%	3.4	0.2	65%	88%

≥ 4	11.9 kPa	82%	96%	19.0	0.2	86%	94%
≥ 5	12.0 kPa	93%	93%	14.0	0.1	74%	99%

SENS: sensitivity; SPEC: specificity; +LR: positive likelihood ratio; -LR: negative likelihood ratio; PPV: positive predictive value; NPV: negative predictive value

Overall, 7 patients with recurrent hepatitis C were misclassified by the 11.9 kPa cut-off with respect to the diagnosis of severe fibrosis. Three S4 and one S5 patients had lower than 11.9 kPa TE values: all these patients had previously received antiviral therapy and none of them had greater than G5. Two S1 and one S3 patients had higher than 11.9 kPa TE results: one had superimposed histological features of acute hepatitis C, whereas the other two had overlapping features of late rejection.

Follow-up study

Forty patients with recurrent hepatitis C underwent sequential paired LB and TE examinations twice. The second examination with paired LB and TE was performed 6 months from the baseline examination in 21 patients, 12 months in 16 patients, 18 months in 2 patients and 21 months in one patient. The initial ranges of grading (1 to 11; median 7) and staging (0 to 5; median 2) changed to 1 to 13 (median 6) and 1 to 6 (median 2) ($p=0.76$ and $p=0.19$, respectively). TE changed from 4.0 to 20.2 kPa (median 7.3 kPa) to 4.1 to 27.1 kPa (median 8.1 kPa) in parallel ($p=0.70$). Changes in histological grading and staging positively correlated with percent changes in TE value ($r=0.63$, $p<0.0001$ and $r=0.71$, $p<0.0001$, respectively). Twelve patients (30%) with increased fibrosis stage showed increased TE values, whereas 24 patients (60%) with decreased or stable fibrosis stage showed equally decreased or stable TE values. Four patients (10%) showed discordant results (Figure 3). TE values were stable according to our criteria despite fibrosis stage increasing from 1 to 2 in one non responder to antiviral therapy patient, who showed improved grading from 9 to 5, and from 1 to 3 in another one patient. In the latter, the first liver biopsy six months following LT showed features of both acute hepatitis and late acute rejection, which disappeared in the second liver biopsy which showed development of chronic hepatitis with bridging fibrosis. In 2 patients (5%), TE values increased from baseline despite decreased or stable scores of fibrosis. One patient with stable staging, showed an ALT flare associated with increased grading from 8 to 13, whereas another one developed de novo AIH / early chronic rejection while responding to antiviral treatment. Six additional patients who were treated with pegylated interferon and ribavirin between the first and second set of examinations, have had an end of treatment response accompanied by stable fibrosis stage and TE results in 3 and decreased fibrosis score and TE results in the remaining 3. Overall, the sensitivity and specificity of TE in predicting worsening of fibrosis stage were 86% and 92%, respectively (Table 6).

Table 6. Performance of transient elastography (TE) in the detection of one point of staging score increase during follow-up.

STAGING	CUT-OFF	SENS	SPEC	+LR	-LR	PPV	NPV
≥ 1 point increase	≥ 30% of TE baseline value increase	86%	92%	11.1	0.15	86%	92%

SENS: sensitivity; SPEC: specificity; +LR: positive likelihood ratio; -LR: negative likelihood ratio; PPV: positive predictive value; NPV: negative predictive value

DISCUSSION

This is the first study to provide data on sequential TE examinations of patients with recurrent hepatitis C. TE accurately predicted severity of graft disease in patients examined both at enrollment and during follow-up. In analogy with non transplanted patients,[13] LT patients with recurrent hepatitis C showed a strong correlation between TE results and histological scores of liver fibrosis, together with high rates (95%) of applicability, i.e. the proportion of patients with a successful examination. However, as in the case of the non-transplant setting,[6,7] in LT patients with fibrosis score ranging from S0 to S4 TE results showed substantial overlap, thus preventing accurate prediction of fibrosis stage on an individual level. Noteworthy, from a clinical point of view, however, was the accuracy in predicting severe bridging fibrosis and cirrhosis (S5 and S6) of the 11.9 kPa cut-off value, which yielded a mere 7% false positive results together with 9% false negative results. The 11.9 kPa cut-off also showed an excellent exclusion ability, as demonstrated by the low (1%) post-test probability of severe fibrosis in patients with lower TE values. Our data therefore are in line with previous observations,[11] indicating TE as having greater negative than positive predictive power for diagnosis of advanced fibrosis. It should be pointed out, however, that the positive predictive power of TE could be biased by histological determinants of liver disease severity that are not represented by Ishak score for fibrosis, like necroinflammatory activity and perisinusoidal fibrosis. As a matter of fact, in our patients, necroinflammatory activity not only positively correlated with TE results, but also it turned out to be an independent predictor of TE results. The importance of histological activity as a moderator of TE is largely disputed. At least four studies in non LT patients [5-7,10], negated a correlation between necroinflammatory activity and TE in patients with liver disease of various etiologies, whereas another study [13] demonstrated a clear-cut correlation between histological necroinflammation and TE results. In at least two of the former studies,[5,7] however, the significance of these correlation studies could have been weakened by lack of chronological concordance between TE examinations and LB, particularly if spontaneous fluctuations of liver cell inflammation had occurred between the exams.[26,27] Discrepancies among studies could also be ascribed to the use of different scoring systems for assessing fibrosis and liver cell inflammation. The Ishak score[23] adopted by us includes four classes of cell necrosis and/or inflammation, each having four to six points of histological damage, compared to the METAVIR score[28] used by others, which consists of four single point classes of histological activity, only. In light of our initial findings, we think that the potential role of perisinusoidal fibrosis as a influencer of TE should be further investigated, since it is a determinant of portal hypertension in LT patients.[29] In our study, perisinusoidal fibrosis emerged as an influencer of TE values at univariate analysis, only. Steatosis, which is widely recognized in the non-transplant setting to be associated with hepatitis C deterioration[30,31], did not appear to influence TE results in our setting. Conversely, high γGT serum levels did emerge as an independent influencer of TE results in our patients maybe as a signal of graft rejection, biliary complications and cholestatic or advanced liver disease.[32-34]

Discrepancies between previous studies and ours could also depend on differences in the yield of liver biopsies, since the sample size of tissue cores greatly influences the estimates of liver fibrosis.[35,36] In our study, the median length of liver cores was

32 mm, with 92% of the specimens being equal or longer than 20 mm, and none was shorter than 15 mm, which is the minimal length considered adequate for confident staging of liver fibrosis.[35-38] In previous studies,[6-11] the median length of liver cores was between 16 mm and 20 mm and, in one study,[12] one third of the biopsy cores was ≤15 mm in length.

Finally, we found that APRI was not able to predict fibrosis in LT patients with recurrent hepatitis C as shown by the less than 0.60 AUROC for the diagnosis of liver fibrosis stage. Likely, changes in platelet counts due to splenomegaly persisting after transplant and fluctuations in serum AST due to LT-related co-morbidities could attenuate the diagnostic accuracy of the serological test for liver fibrosis. Our data contrast with a previous study of 51 patients with recurrent hepatitis C, in whom APRI did predict the degree of graft fibrosis during follow-up.[39] However, the conclusions of that study need to be re-examined following expansion of the sample size and elimination of repeated examinations of the same patients with less impaired platelet count.

The strength of our study was the concurrent TE and protocol LB examinations in patients with recurrent hepatitis C who were prospectively followed-up, and that an increase of TE values predicted deterioration of liver disease at LB while protocol LB in patients with decreased or stable TE results demonstrated unchanged histological lesions compared to baseline.

In conclusion, we think that TE can reliably assess fibrosis progression in LT patients with recurrent hepatitis C and that, therefore, it might become an adjunct to LB in the management of LT patients, thereby reducing the need for protocol LB in the evaluation of hepatitis progression.

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Statement of competing interests: None to declare.

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FIGURE LEGENDS

Figure 1. Box plots of transient elastography (TE) results for each fibrosis stage. The top and the bottom are the first and third quartiles. The length of the box represents interquartile ranges, within which 50% of the values are located. The lines through the middle of the boxes represent median values of TE for each fibrosis stage. TE values were significantly different for each fibrosis stage ($p<0.0001$). Below the graph is reported the number of patients for each fibrosis stage.

Figure 2. ROC curves for transient elastography (TE) results for different fibrosis stage thresholds. The ROC curve is a plot of sensitivity versus 1-specificity for all

possible cut-off values. AUROC is the area under ROC curve. The figure shows ROC curves constructed for the detection of patients with S≥2, S≥3 and S≥4.

Figure 3. Changes in transient elastography (TE) results and staging score in the 40 patients with recurrent hepatitis C who underwent sequential examinations with paired liver biopsy and TE during follow-up. Graphs represent the four groups of patients according to concordance or discordance between TE and liver histology: 12 patients (30%) with increased fibrosis stage and increased TE results **(A)**, 24 patients (60%) with decreased or stable fibrosis stage and equally decreased or stable TE values **(B)**, 2 patients (5%) with decreased or stable fibrosis stage and increased TE values **(C)** and 2 patients (5%) with increased fibrosis stage and stable TE results **(D)**.

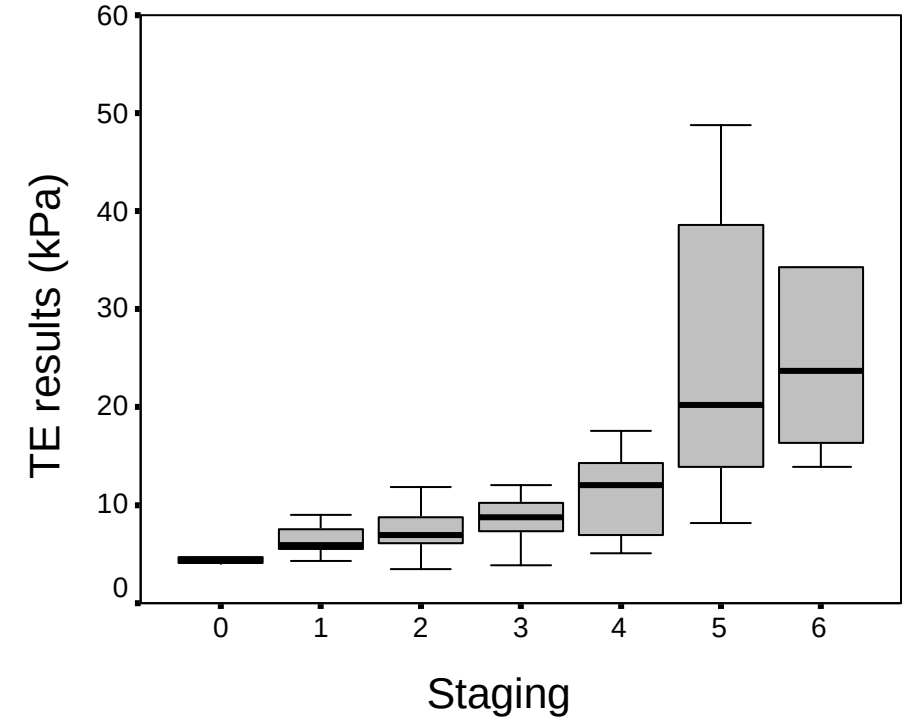
REFERENCES

1 Rockey DC, Bissell DM. Noninvasive measures of liver fibrosis. *Hepatology* 2006;**43**:S113-S120.
2 Piccinino F, Sagnelli E, Pasquale G *et al*. Complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies. *J Hepatol* 1986;**2**:165-73.
3 Afdhal NH, Curry M. Technology evaluation: a critical step in the clinical utilization of novel diagnostic tests for liver fibrosis. *J Hepatol* 2007;**46**:543-5.
4 Burroughs AK, Cholongitas E. Non-invasive tests for liver fibrosis: Encouraging or discouraging results? *J Hepatol* 2007;**46**:751-5.
5 Sandrin L, Fourquet B, Hasquenoph JM *et al*. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003;**29**12:1705-13.
6 Castera L, Vergniol J, Foucher J *et al*. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005;**128**:343-50.
7 Ziol M, Handra-Luca A, Kettaneh A *et al*. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005;**41**:48-54.
8 Foucher J, Chanteloup E, Vergniol J *et al*. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut* 2006;**55**(3):403-8.
9 de Ledinghen V, Douvin C, Kettaneh A *et al*. Diagnosis of hepatic fibrosis and cirrhosis by transient elastography in HIV/hepatitis C virus-coinfected patients. *J Acquir Immune Defic Syndr* 2006;**41**:175-9.
10 Corpechot C, El Naggar A, Poujol-Robert A *et al*. Assessment of biliary fibrosis by transient elastography in patients with PBC and PSC. *Hepatology* 2006;**43**:1118-24.
11 Ganne-Carrie N, Ziol M, de Ledinghen V *et al*. Accuracy of liver stiffness measurement for the diagnosis of cirrhosis in patients with chronic liver diseases. *Hepatology* 2006;**44**:1511-7.
12 Kettaneh A, Marcellin P, Douvin C *et al*. Features associated with success rate and performance of FibroScan measurements for the diagnosis of cirrhosis in HCV patients: a prospective study of 935 patients. *J Hepatol* 2007;**46**:628-34.
13 Fraquelli M, Rigamonti C, Casazza G *et al*. Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. *Gut* 2007;**56**:968-73.

14 Angelico M, Gridelli B, Strazzabosco M. Practice of adult liver transplantation in Italy. Recommendations of the Italian Association for the Study of the Liver (A.I.S.F.). *Dig Liver Dis* 2005;**37**:461-7.
15 Ray RA, Lewin KJ, Colonna J *et al*. The role of liver biopsy in evaluating acute allograft dysfunction following liver transplantation: a clinical histologic correlation of 34 liver transplants. *Hum Pathol* 1988;**19**:835-48.
16 Hubscher SG. Transplantation pathology. *Semin Diagn Pathol* 2006;**23**:170-81.
17 Carrion JA, Navasa M, Bosch J *et al*. Transient elastography for diagnosis of advanced fibrosis and portal hypertension in patients with hepatitis C recurrence after liver transplantation. *Liver Transpl* 2006;**12**:1791-8.
18 Ferrell LD, Wright TL, Roberts J *et al*. Hepatitis C viral infection in liver transplant recipients. *Hepatology* 1992;**16**:865-76.
19 Czaja AJ. Autoimmune hepatitis after liver transplantation and other lessons of self-intolerance. *Liver Transpl* 2002;**8**:505-13.
20 Demetris AJ, Adeyi O, Bellamy CO *et al*. Liver biopsy interpretation for causes of late liver allograft dysfunction. *Hepatology* 2006;**44**:489-501.
21 Brunt EM, Janney CG, Di Bisceglie AM *et al*. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999;**94**:2467-74.
22 Wai CT, Greenson JK, Fontana RJ *et al*. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003;**38**:518-26.
23 Ishak K, Baptista A, Bianchi L *et al*. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;**22**(6):696-9.
24 Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology* 1997;**25**:658-63.
25 Demetris AJ, Eghtesad B, Marcos A *et al*. Recurrent hepatitis C in liver allografts: prospective assessment of diagnostic accuracy, identification of pitfalls, and observations about pathogenesis. *Am J Surg Pathol* 2004;**28**:658-69.
26 Pontisso P, Bellati G, Brunetto M *et al*. Hepatitis C virus RNA profiles in chronically infected individuals: do they relate to disease activity? *Hepatology* 1999;**29**:585-9.
27 Rumi MG, De Filippi F, La Vecchia C *et al*. Hepatitis C reactivation in patients with chronic infection with genotypes 1b and 2c: a retrospective cohort study of 206 untreated patients. *Gut* 2005;**54**:402-6.
28 Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996;**24**:289-93.
29 Blasco A, Forns X, Carrion JA *et al*. Hepatic venous pressure gradient identifies patients at risk of severe hepatitis C recurrence after liver transplantation. *Hepatology* 2006;**43**:492-9.
30 Castera L, Hezode C, Roudot-Thoraval F *et al*. Worsening of steatosis is an independent factor of fibrosis progression in untreated patients with chronic hepatitis C and paired liver biopsies. *Gut* 2003;**52**:288-92.
31 Fartoux L, Poujol-Robert A, Guechot J *et al*. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. *Gut* 2005;**54**:1003-8.

32 Shastri YM, Hoepffner NM, Akoglu B *et al*. Liver biochemistry profile, significance and endoscopic management of biliary tract complications post orthotopic liver transplantation. *World J Gastroenterol* 2007;**13**:2819-25.
33 Hickman PE, Lynch SV, Potter JM *et al*. Gamma glutamyl transferase as a marker of liver transplant rejection. *Transplantation* 1994;**57**:1278-80.
34 Nery J, Esquenazi V, Weppler D *et al*. Hepatitis C in liver transplantation: preliminary study of prognostic factors. *Transpl Int* 1994;**7**:S229-S231.
35 Colloredo G, Guido M, Sonzogni A *et al*. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. *J Hepatol* 2003;**39**:239-44.
36 Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003;**38**:1449-57.
37 Guido M, Rugge M. Liver biopsy sampling in chronic viral hepatitis. *Semin Liver Dis* 2004;**24**:89-97.
38 Cholongitas E, Senzolo M, Standish R *et al*. A systematic review of the quality of liver biopsy specimens. *Am J Clin Pathol* 2006;**125**:710-21.
39 Toniutto P, Fabris C, Bitetto D *et al*. Role of AST to platelet ratio index in the detection of liver fibrosis in patients with recurrent hepatitis C after liver transplantation. *J Gastroenterol Hepatol* 2007;**22**:1904-8.

Figure 1.



Patients No.	5	25	28	10	7	9	6

Figure 2.

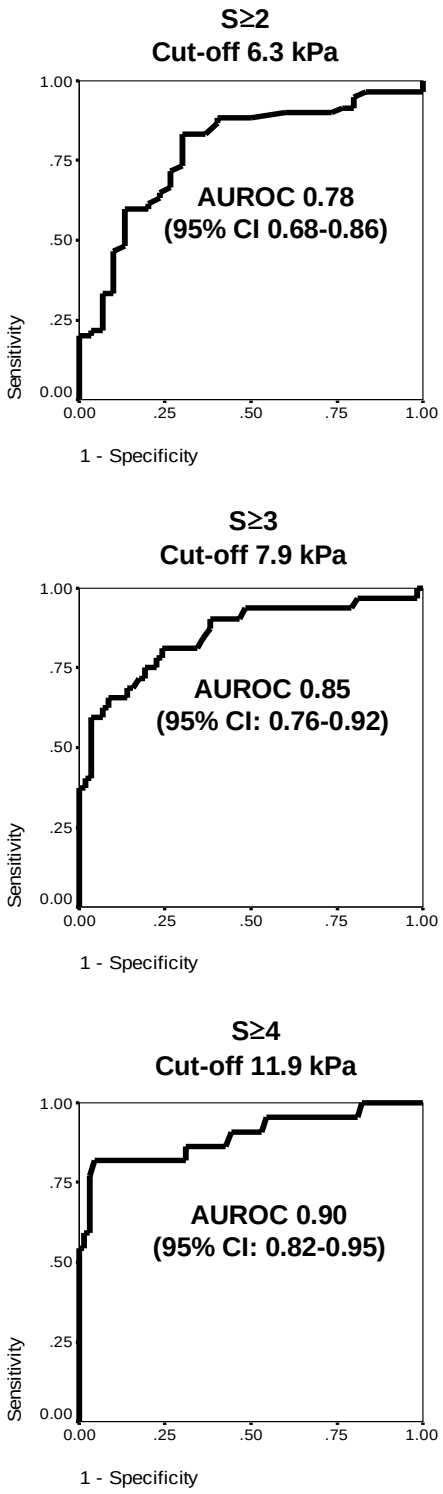
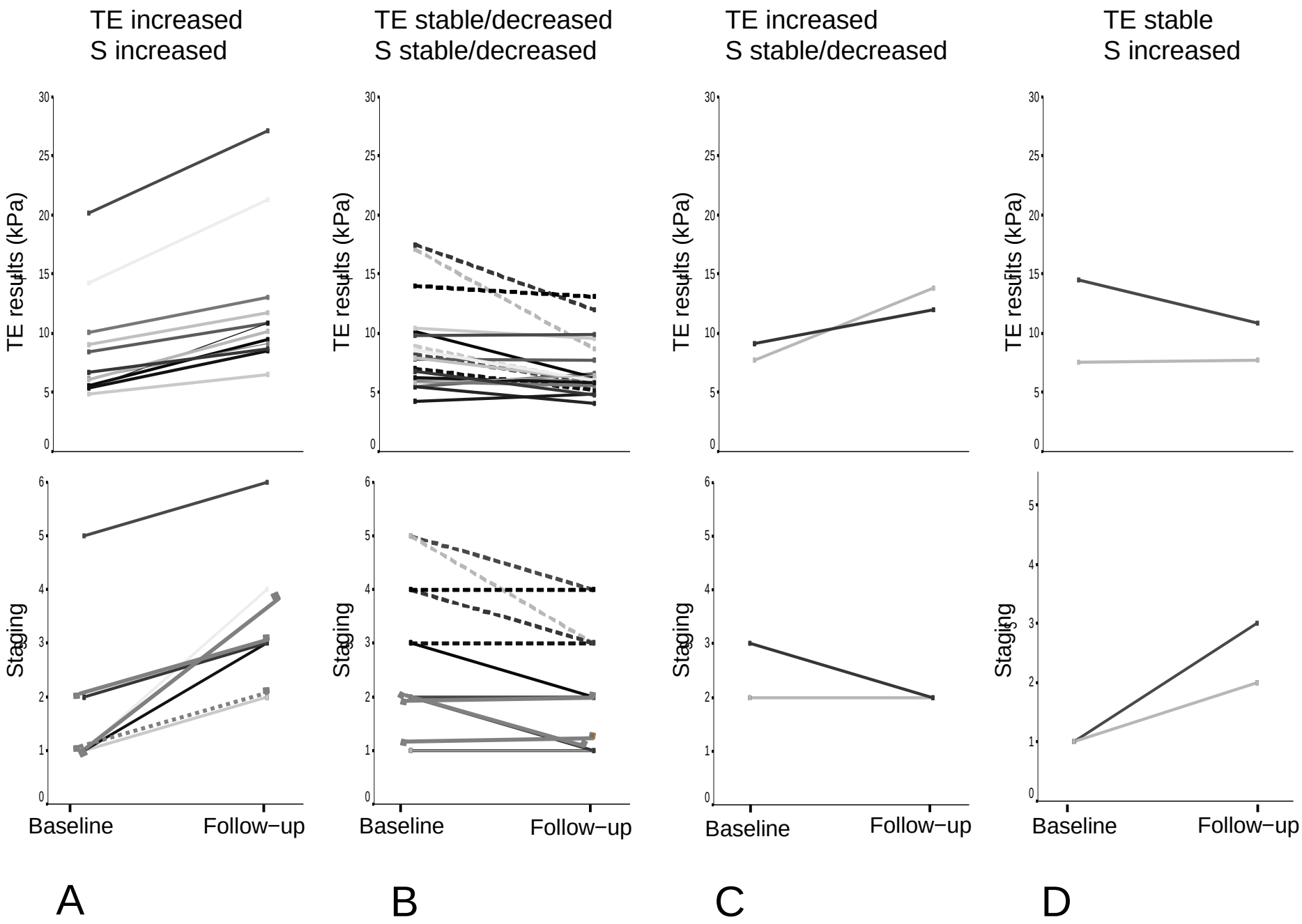


Figure 3.



Transient elastography predicts fibrosis progression in patients with recurrent hepatitis c after liver transplantation

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