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Liver Stiffness Is Influenced by a Standardized Meal in Patients With Chronic Hepatitis C Virus at Different Stages of Fibrotic Evolution

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Transient elastography (TE) is increasingly employed in clinical practice for the noninvasive detection of tissue fibrosis in patients with chronic liver disease (CLD), and particularly chronic hepatitis C virus (HCV)-related hepatitis. The present study was designed to provide a definitive characterization of the "confounding" increase in liver stiffness (LS) following a standardized meal in a consecutive population of 125 patients with chronic HCV infection at different stages of fibrotic evolution. LS values were obtained after overnight fasting and 15, 30, 45, 60, and 120 minutes following the onset of a standardized liquid meal (400 mL, 600 Kcal, 16.7% protein, 53.8% carbohydrates, 29.5% fat). An evident increase in LS values was observed 15 to 45 minutes after the onset of the meal with return to baseline premeal levels within 120 minutes in all patients. The peak postmeal delta increase in LS was progressively more marked with increasing stages of fibrosis ($P < 0.001$), becoming maximal in patients with cirrhosis. However, the probability of identifying the Metavir stage of fibrosis, the Child-Pugh class, or the presence/absence of esophageal varices with the postmeal delta increase in LS was inferior to that obtained with baseline LS values. *Conclusion*: The results of the present study provide definitive evidence of the confounding effect of a meal on the accuracy of LS measurements for the prediction of fibrosis stage in patients with chronic HCV hepatitis and suggest that a fasting period of 120 minutes should be observed before the performance of TE. (HEPATOLOGY 2013;58:65-72)

Transient elastography (TE) is increasingly employed in clinical practice for the noninvasive detection of tissue fibrosis in patients with chronic liver disease (CLD), and particularly chronic hepatitis C virus (HCV)-related hepatitis.[1] In this clinical setting, TE has been shown to be able to discriminate between at least three stages of fibrotic evolution: the absence of significant fibrosis, the presence of advanced fibrosis/cirrhosis, and an intermediate stage, often defined as a "gray area." This distinction is useful in everyday practice for directing the need of liver biopsy,[2] and overall, the use of TE, alone or in association with other noninvasive means, considerably reduces the number of liver biopsies necessary for correct patient management.

It is increasingly clear that factors such as significant liver tissue necroinflammation, cholestasis, and tissue congestion[3-6] may affect the assessment of liver stiffness (LS) values obtained by TE, thus leading to an overestimation of the stage of liver fibrosis. The identification of additional "confounding factors" is relevant for a more accurate use of TE in patients with chronic liver disease. Along these lines, Mederacke et al.[7] reported a significant increase in LS values immediately after a nonstandardized meal and up to 60 minutes followed by normalization after 180 minutes in a group of patients with chronic or resolved HCV infection without histopathological assessment of disease stage. As

Abbreviations: CLD, chronic liver disease; LS, liver stiffness; PBF, portal blood flow; TE, transient elastography.

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suggested by the authors, this potentially confounding increase in LS values is likely due to an increased rigidity of liver tissue consequent to a physiological response defined as "postprandial hyperemia."[8,9] This observation is relevant since, due to the expansion of TE in clinical practice, measurements of LS values are obtained during the whole working day and, therefore, in patients with a potentially insufficient fasting period.

The aim of the present study was to provide an accurate characterization of the "confounding" increase in LS following a standardized meal in a consecutive population of 125 patients with chronic HCV infection at different stages of fibrotic evolution.

Patients and Methods

Patients. One hundred twenty-five consecutive patients with HCV-related chronic liver disease (CLD) (57 men and 68 women, age 20 to 78 years) referred between March 2009 and April 2010 to the clinical hepatology services of the Azienda Ospedaliera Universitaria Careggi (AOUC), Florence, Italy (35 patients), of the 3rd Medical Clinic, University of Medicine and Pharmacy Cluj-Napoca, Romania (73 patients), and of the IRCCS "Casa Sollievo della Sofferenza" San Giovanni Rotondo, Foggia, Italy (17 patients) for the assessment of disease stage aimed at a possible antiviral treatment were included in the study.

Inclusion criteria were: abnormal levels of liver enzymes and the presence of detectable HCV-RNA. Exclusion criteria were: the presence of ascites at clinical or ultrasound examination, the presence of hepatocellular carcinoma, acute liver disease, or coinfection with HBV or HIV, metabolic liver disease, autoimmune hepatitis, vascular disease of the liver, biliary tract disorders, and treatment with antiviral drugs. The presence of alcohol abuse or the use of hepatotoxic drugs within 6 months preceding the study was excluded in all patients. In addition, clinical conditions potentially affecting TE, e.g., cardiac failure, or in which this technique is contraindicated, e.g., pregnancy, were also excluded.

Based on clinical (history, physical findings), laboratory evidence (hypoalbuminemia, hyperbilirbinemia, low platelet count, increased international normalized ratio, INR) sonographic (irregular liver edge, inhomogeneous coarse echo pattern, ratio of caudate lobe to right lobe >0.6, increased portal vein diameter, splenomegaly), and endoscopic (presence of esophageal varices) criteria, 31 patients out of 125 were classified as frankly "cirrhotic" and were included in the study protocol without liver biopsy. At least two criteria from each group were required to establish the diagnosis of cirrhosis. Liver biopsy was performed in the remaining 94 patients. On the same study day, patients underwent a complete ultrasound examination of the upper abdomen, measurement of liver stiffness by TE, and ultrasound-guided percutaneous liver biopsy. The following clinical and biological parameters were determined on the study day: body mass index (BMI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), Y-glutamyl-transpeptidase (GGT), total bilirubin, platelet count, INR, albumin, glucose, and creatinine.

In addition to the initial 31 patients classified as "cirrhotic," the diagnosis of cirrhosis (Metavir F4) was based on the results of the liver biopsy performed at the time of the study in an additional nine patients, for a total of 40 patients in Metavir stage F4. All these patients underwent upper gastrointestinal (GI) endoscopy within 1 week from the study day in order to assess the presence of esophageal varices, congestive gastropathy, and other abnormalities related to portal hypertension.

The nature of the study was explained to all patients, each of whom provided written informed consent before the beginning of the study, in accordance with the principles of the Declaration of Helsinki (revision of Edinburgh, 2000).

The major clinical and biochemical parameters of the patients included in the study stratified by the Metavir stage of liver fibrosis (F0-F1, F2-F3, and F4) are listed in Table 1.

Transient Elastography. TE was performed after a complete ultrasound examination of the upper abdomen using the FibroScan apparatus (Echosens, Paris, France) as described.[9] Measurements were performed after an overnight fasting (baseline values) and 15, 30, 45, 60, and 120 minutes following the intake of a standardized liquid meal (Ensure Plus Drink, Abbot

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Table 1. Clinical Parameters of the 125 Patients Included in the Study, Stratified by Liver Fibrosis Stage

	Normal Range	F0-1 (n = 50) Median	IQR	F2-3 (n = 35) Median	IQR	F4 (n = 40) Median	IQR	JT test *P* Value
Sex (M/F)		(17/33)		(19/16)		(20/20)		
Age (years)		43	19	57	20	58	17	<0.001
ALT (U/L)	5-40	45	40	67	45.5	71	52	<0.001
AST (U/L)	5-40	35	20	55	31	65	56	<0.001
Bilirubin (mg/dL)	0.30-1	0.6	0.2	0.7	0.4	1.1	0.5	<0.001
GGT (U/L)	10-40	33	22	68	67	64	60	<0.001
INR (sec)	0.8-1.2	1.0	0.1	1.0	0.2	1.1	0.4	<0.001
Platelets (x $10^3/mm^3$)	140- 440	222	64	165	99	115	57	<0.001
BMI (kg/m^2)	18.5-24.9	24.8	2.8	26.4	4.8	24.5	5.1	0.740
Creatinine (mg/dL)	0.48-1.03	0.9	0.1	1.0	0.2	0.9	0.2	0.933
Fasting Glucose (mg/dL)	65-110	91	15	92	14	90	24	0.247
Albumin (g/L)	4.20-4.76	4.2	0.5	4.0	0.6	3.6	0.9	<0.001

Abbreviations: BMI, body mass index; F0-F4, METAVIR stage of fibrosis; IQR, interquartile range; JT, Jonckheere-Terpstra test.

Laboratories, Zwolle, the Netherlands): 400 mL, 600 Kcal, 16.7% protein, 53.8% carbohydrates, 29.5% fat. The ingestion of the meal was achieved within a maximum period of 5 minutes in all patients, and the onset of the ingestion was taken as time zero. Since the upright posture has been reported to blunt postprandial hyperemia in patients with cirrhosis,[10] LS measurements were performed maintaining all patients in the recumbent position after the onset of the meal.

Before the beginning of the study, 400 mL of water were administered to 20 patients (all from AOUC) with HCV-related chronic liver disease (F0: n = 3, F1: n = 5, F2: n = 5, F3: n = 3, F4: n = 4) and LS measurements were performed at the same timepoints (baseline, 15, 30, 45, 60, 120 minutes) in order to evaluate potential artifacts due the administration of an identical liquid volume. In all patients of this test cohort no appreciable variations in LS values were observed following the assumption of the liquid volume (Table 2).

All TE operators were staff physicians who had previously performed at least 500 determinations in patients with chronic liver disease. As described[3] and as suggested by the provider of the instrumentation, we considered representative measurements the median value of 10 successful acquisitions with a success rate of at least 60%, and with an interquartile range (IQR) over median ratio lower than 30%.

Ultrasound-Assisted Liver Biopsy. The clinical indication for liver biopsy was to assess the evolution of chronic viral disease in patients with viral infection. The biopsy was performed under ultrasound guide on the right lobe of the liver with a 16G semiautomatic modified Menghini needle system (BIOMOL; Hospital Service, Aprilia, Italy) under local anesthesia. Before the procedure and after an overnight fast, the patients received 5 mg of diazepam and 5 mg of atropine. Liver specimens were formalin-fixed and paraffin-embedded for histological evaluation. Sections of liver tissue, 5-μm-thick, were stained with hematoxylin/eosin and Masson trichrome, and examined by experienced pathologists, blinded to the results of LSM and clinical data. Only liver specimens with a length >25 mm and including at least 11 complete portal tracts were considered adequate for the study.[11] Histological diagnoses were established according to internationally accepted criteria.[12,13] Fibrosis (F) and necroinflammatory activity (A) were evaluated semiquantitatively according to the Metavir scoring system[12]: fibrosis was staged on a 0-4 scale: F0, absence of fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; F4, cirrhosis.

Statistical Analysis. For statistical evaluation the patients included in the study were grouped as follows: F0-F1 (absence and minimal fibrosis), F2-F3

Table 2. Baseline and Postwater Liver Stiffness Values in the 20 Patients Undergoing the Test Stratified by Liver Fibrosis Stage

	F0-1 (n = 8) Median	IQR	F2-3 (n = 7) Median	IQR	F4 (n = 5) Median	IQR	JT test *P* Value
S_0 (kPa)	5.3	0.5	9.0	0.7	17.8	1.3	<0.001
S_{15} (kPa)	5.5	0.6	9.2	0.9	18.0	1.5	<0.001
S_{30} (kPa)	5.3	0.6	9.3	0.7	17.7	1.1	<0.001
S_{45} (kPa)	5.4	0.5	9.1	0.8	17.9	0.9	<0.001
S_{60} (kPa)	5.3	0.4	9.1	1.0	18.1	1.0	<0.001
S_{min} (kPa)	5.3	0.4	9.0	0.7	17.7	1.1	<0.001
S_{max} (kPa)	5.5	0.6	9.2	0.8	18.1	1.0	<0.001
S_{delta} (kPa)	0.2	0.2	0.3	0.7	0.4	0.6	<0.001
S_{delta} (%)	3.7	20.0	3.3	14.2	0.2	15.3	<0.001

Abbreviations: F0-F4, METAVIR stage of fibrosis; IQR, interquartile range; JT, Jonckheere-Terpstra test; S_0-S_{60}, stiffness values at different time points during the meal test; S_{min}, minimal stiffness value during the meal test; S_{max}, maximal stiffness value during the meal test; S_{delta} (kPa), difference between maximal and minimal stiffness value during the test meal; S_{delta} (%), [(maximal stiffness basal stiffness) / basal stiffness] x 100.

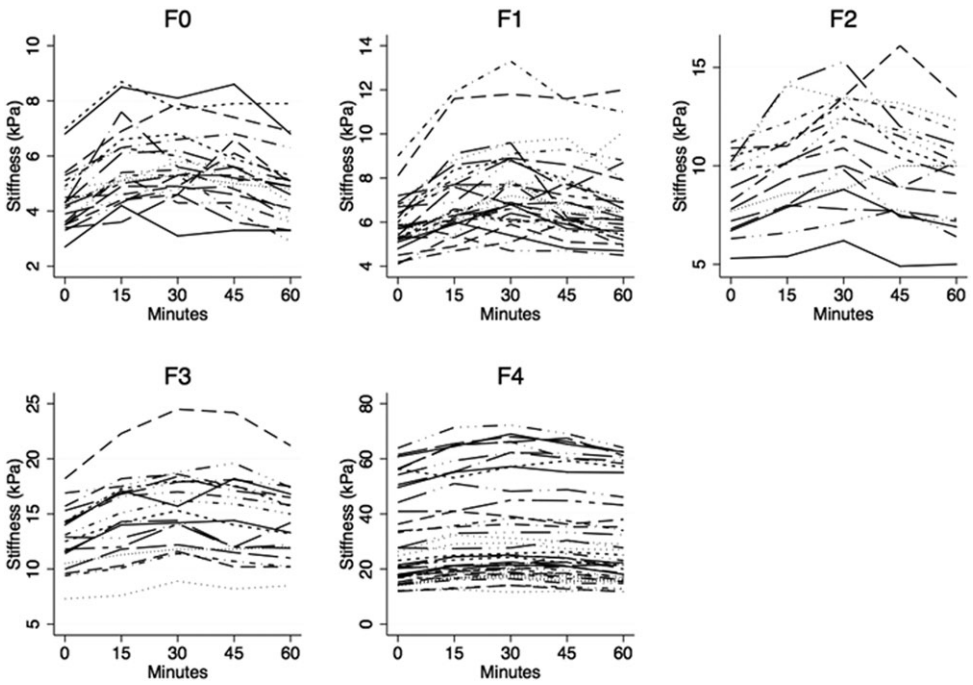

Fig. 1. Individual trajectories of liver stiffness values during the meal test in the 125 patients included in the study stratified by individual Metavir stages of liver fibrosis. Please note that different scales of values have been used on the *y* axis in order to highlight the peak of each trajectory.

(significant-advanced fibrosis), F4 (cirrhosis). Descriptive statistics are reported as median and IQR because most of the variables, especially stiffness, were not normally distributed. IQR was calculated as the difference between the 75th and 25th percentile. Between-group comparisons of continuous variables were performed using the asymptotic or exact version of the Wilcoxon-Mann-Whitney test according to the available sample size. The Jonckheere-Terpstra test for ordered alternatives was used to test the existence of a trend between the stage of liver fibrosis and the stiffness parameters.[14] An ordinal generalized logistic regression model (OGLM) was used to evaluate the association of S_0 (kPa) and S_{delta} (kPa) with the stage of fibrosis. The OGLM allows directly controlling for heteroscedasticy and defaults to the proportional odds logistic regression model when the parallel-lines assumption is met.[15] The odds ratio obtained from such an ordinal model is a measure of the odds of more severe versus less severe fibrosis. Probabilities estimated from the OGLM were plotted to aid the clinical interpretation of the results. Besides evaluating probability curves, we compared the prediction of liver fibrosis obtained by S_0 and S_{delta} using the Bayesian information criterion (BIC).[16] Statistical significance was set to $P < 0.05$ and all statistical tests were two-tailed. Statistical analysis was performed using Stata 12.1 (Stata Corp, College Station, TX) together with the user-written OGLM package.[15]

Results

Characteristics of Patients. As mentioned, inclusion in the F4 group (40 patients) derived either from histopathological staging at the time of the study or on clinical, laboratory, sonographic, and endoscopic parameters. In this group, 27 patients out of 40 were classified as Child-Pugh A, whereas 13 were classified as Child-Pugh B. The presence of esophageal varices (OV) was detected in 20 out of 40 patients, eight in the Child-Pugh A group (four OV grade 1, four OV grade 2) and in 12 in the Child-Pugh B group (four OV grade 1, eight OV grade 2).

Changes of Liver Stiffness Values After a Standardized Meal. In the absence of previous data precisely indicating the exact time of LS postmeal peak increase, LS measurements were performed 15, 30, 45, 60, and 120 minutes after the onset of the meal. Figure 1 illustrates the individual changes of stiffness following the onset of the meal test in the whole patient population according to the degree of fibrosis. Although most patients, irrespective of the stage of fibrosis, presented a peak increase after 30 minutes, some variability was observed, with some patients peaking at 15 or 45 minutes. Values returned to baseline levels within 120 minutes in all patients independently of the stage of fibrosis.

As illustrated in Table 3, changes in liver stiffness were evaluated by means of the following continuous indexes: S_0 = baseline value of stiffness, $S_{15\text{-}60}$ = values at 15, 30, 45, and 60 minutes during the meal

Table 3. Baseline and Postmeal Liver Stiffness Values in the 125 Patients Included in the Study Stratified by Liver Fibrosis Stage

	F0-1 (n = 50)		F2-3 (n = 35)		F4 (n = 40)		
	Median	IQR	Median	IQR	Median	IQR	JT test *P* Value
S_0 (kPa)	5.0	1.4	10.7	3.4	21.2	25.7	<0.001
S_{15} (kPa)	5.9	1.7	12.2	4.3	24.5	27.3	<0.001
S_{30} (kPa)	6.2	1.8	14.2	5.1	24.9	27.3	<0.001
S_{45} (kPa)	5.7	1.4	12.1	5.0	24.9	28.4	<0.001
S_{60} (kPa)	5.5	1.3	11	4.2	22.7	27.7	<0.001
S_{min} (kPa)	5.0	1.4	10.7	3.4	21.2	25.9	<0.001
S_{max} (kPa)	6.7	1.9	13.2	5.0	25.4	28.7	<0.001
S_{delta} (kPa)	1.9	0.9	2.7	0.8	4.7	2.8	<0.001
S_{delta} (%)	33.6	21.1	25.3	8.6	16.6	7.5	<0.001

Abbreviations: F0-F4, METAVIR stage of fibrosis; IQR, interquartile range; JT, Jonckheere-Terpstra test; S_0-S_{60}, stiffness values at different time points during the meal test; S_{min}, minimal stiffness value during the meal test; S_{max}, maximal stiffness value during the meal test; S_{delta} (kPa), difference between maximal and minimal stiffness value during the test meal; S_{delta} (%), [(maximal stiffness basal stiffness) / basal stiffness] x 100.

test, respectively; S_{min} = minimum value of stiffness, S_{max} = maximum value of stiffness, S_{delta} (kPa) = (maximal stiffness − basal stiffness), S_{delta} (%) = (maximal stiffness − basal stiffness) / basal stiffness × 100. With the exception of S_{delta} (%), which showed a decreasing trend, all stiffness indexes showed an increasing trend for increasing stages of fibrosis ($P < 0.0001$ for all, Jonckheere-Terpstra test), as also illustrated in Fig. 2 for S_{delta} (kPa).

Probability of Detecting the Stage of Fibrosis at Different Timepoints After a Standardized Meal. Since most centers do not apply a fasting time before the TE procedure, the probability of detecting fibrosis stage at each timepoint: basal, 15, 30, 45, and 60 minutes postmeal was evaluated (Fig. 3). It is evident from the comparison of the probability curves that no other timepoint was superior than S_0 in detecting any stage of fibrosis.

The same analysis was applied to the comparison of basal stiffness and delta stiffness based on the peak change irrespective of the postmeal timepoint. Figure 4 illustrates the probability (point estimate and 95% confidence intervals) of fibrosis stage (F0-F1, F2-F3, andF4) on the basis of S_0 (kPa) and S_{delta} (kPa). Values of S_0 ≥18 kPa were associated with a probability of F4 fibrosis ≥92%, confirming our previous observations in a different series of patients with HCV-induced CLD.[3] Even if the probability of fibrosis was also associated with S_{delta} (kPa), this latter was clearly not superior to S_0 in detecting the probability of liver fibrosis. The worse performance of the prediction based on S_{delta} was also evident by the comparison of its BIC value (332) with that of the S_0 model (179).

Probability of Detecting the Child-Pugh Stage and the Presence of Esophageal Varices From Basal Stiffness and Delta Stiffness After a Standardized Meal. Although the between-group difference in S_{delta} (kPa) was significant (exact-$P = 0.037$), it was not useful for discriminating between Child-Pugh stage A and B because of substantial overlap (data not shown). The same conclusion applies to the ability of S_{delta} (kPa) to discriminate between the presence or the absence of esophageal varices (exact-$P = 0.0009$). In both cases, the use of S_{delta} (kPa) did not show any advantage when compared to S_0 (kPa) (data not shown).

Discussion

During the past decade, TE has been shown to represent an important tool for the assessment of the fibrotic evolution of CLD, particularly chronic HCV hepatitis. In this context, the integration of TE and other noninvasive methods with liver biopsy has brought definite advantages in the allocation of patients in different classes of disease progression.[1,17] Because of the increasing use of TE in the everyday management of patients with chronic HCV hepatitis, major efforts are dedicated to the optimal standardization of this methodology in view of its inclusion in clinical practice guidelines. Along these lines, the identification of factors negatively affecting the diagnostic accuracy of TE, i.e., "confounding factors," is absolutely crucial.

The work by Mederacke at al.[7] highlighted the possibility that LS values may be affected if TE is performed shortly after a meal. Considering that in most centers TE is scheduled during the whole working day and that there are not precise recommendations concerning fasting prior to the performance of TE, overestimation of LS values is likely a frequent occurrence. Even a minor overestimation of 3-4 kPa may have a significant impact on the interpretation of this noninvasive method,

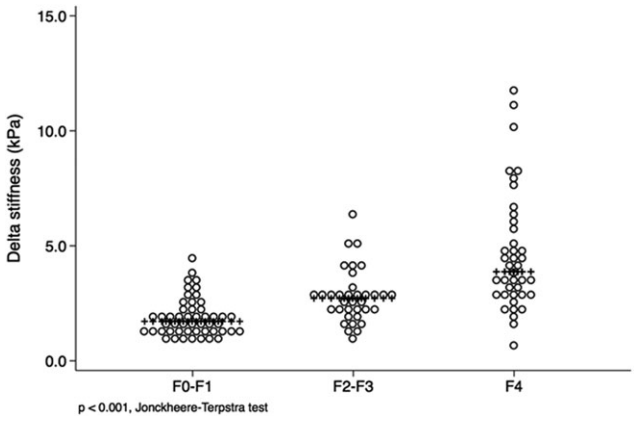

Fig. 2. Relationship between delta stiffness and stage of fibrosis.

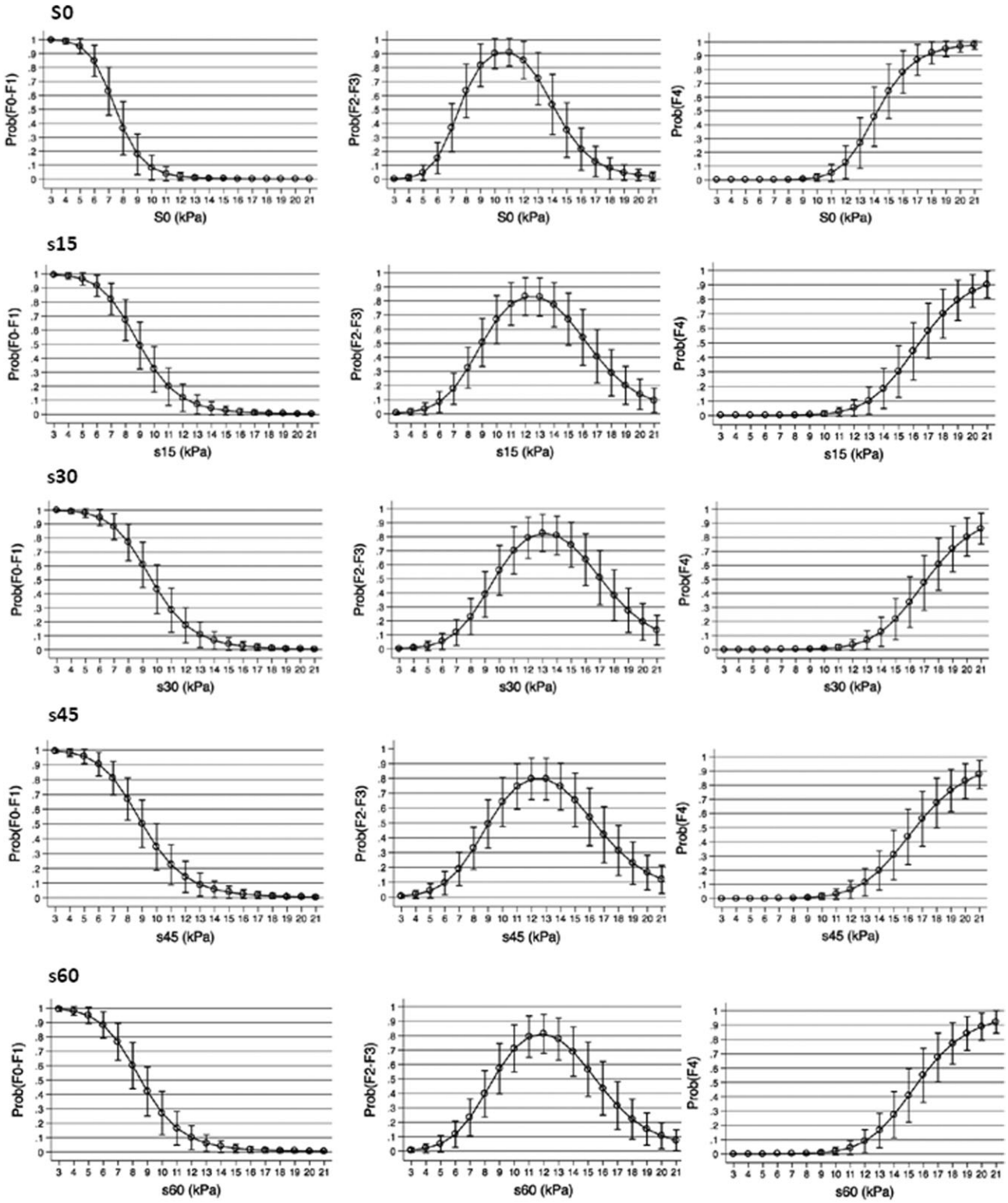

Fig. 3. Probability of fibrosis at each timepoint: basal (s0), 15 (s15), 30 (s30), 45 (s45), and 60 (s60) minutes postmeal. It is evident from the comparison of the probability curves that no other timepoint was superior than s0 in detecting any stage of fibrosis.

especially for the early stages of fibrosis (i.e., F0-F2), thus leading to errors in clinical management when following the proposed flow-charts.[2] In addition, the observation of a "dynamic" change in LS values following a meal offers an additional opportunity in the use of TE, i.e., the possibility to monitor, over a short time frame, dynamic changes in LS values that may differ in their intensity in different stages of fibrotic evolution of the disease.

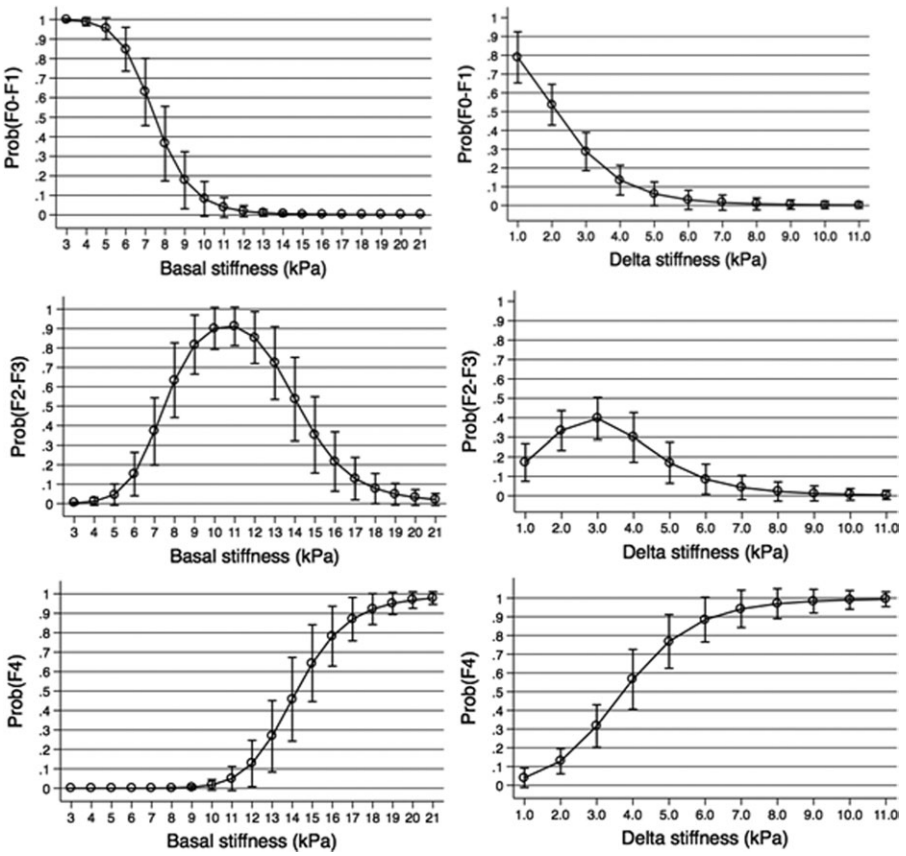

Fig. 4. Probability of fibrosis (point estimate and 95% confidence intervals) on the basis of basal stiffness and postmeal delta stiffness. Values of stiffness >21 were always associated with F4 fibrosis and are not plotted.

Accordingly, the present study was designed in order to overcome the main limitations of the study by Mederacke et al.: patient categorization not supported by histopathological staging but rather based on baseline LS values, lack of standardization in the administration of the test meal (Continental breakfast with ~600 kcal consumed over a maximum period of 30 minutes), and measurement of LS values at timepoints not clearly related to postprandial hyperemia (immediately and 60 minutes after the onset of the meal). Different from the study by Mederacke et al., where the authors did not observe any increase in LS values in patients with baseline values over 10 kPa, a cutoff value that allows predicting significant or advanced fibrosis but not cirrhosis,[3] the results of the present investigation clearly indicate that LS values increase after a standardized meal in patients with chronic HCV infection at any stage of fibrotic evolution and in patients with compensated cirrhosis. The increase in LS, with return to baseline values within 120 minutes, is not just related to the rapid assumption of the liquid volume but rather associated with the overall caloric intake of the meal.

The meal test with postmeal portal blood flow (PBF) measurements has been suggested as a reproducible noninvasive test to evaluate the severity of portal hypertension in cirrhosis patients. The effect of postprandial hyperemia on portal pressure has been reported 30 minutes after the onset of the meal both by direct measurement[18] and by Doppler sonography[19] in cirrhosis patients. Data in normal subjects and in noncirrhosis patients with CLD are scarce and obtained only by Doppler sonography,[20-22] but indicate that an increase in PBF is detectable by Doppler sonography also 30 minutes after the onset of the meal.

Changes in LS values following a test meal are likely a consequence of the adaptation of the hepatic microcirculation to an increased PBF[8,9] and are in overall agreement with the observation that postprandial hyperemia is associated with a greater increase in portal pressure in cirrhosis patients. In this context, the progressive increase in postmeal delta LS values along with the fibrotic evolution of chronic HCV hepatitis could represent an indirect index of the progressive impairment of the mechanisms responsible for this adaptation, particularly sinusoidal circulation autoregulation, as a consequence of tissue fibrosis, inflammatory infiltration, and neoangiogenesis.[23-25]

Overall, these findings highlight an interesting potential of TE in detecting dynamic changes in LS related to both the anatomical modifications and hemodynamic alterations occurring in the progression of chronic HCV hepatitis. Accordingly, we tested whether or not the delta stiffness increase in postmeal LS values had advantages,

when compared with premeal baseline LS values, in assessing the probability of liver fibrosis according to the Metavir staging system. While premeal baseline LS values were rather accurate in defining the probability of fibrosis stage and in agreement with previous observations by our group in a completely different cohort of patients with HCV-induced CLD,[3] an analysis of the performance of the postmeal delta stiffness increase revealed that changes in LS values occurring after the meal test do not offer any advantage in the detection of different stages of fibrosis, whose definition becomes actually less accurate. Although this decrease in accuracy is common to all the stage groups, it is clearly more marked for the group F2-F3, i.e., significant or advanced fibrosis. However, although the changes in LS values elicited by the meal do not offer any advantage in the prediction of the fibrosis stage when compared with premeal baseline values, a peak delta LS increase $\geq$8 kPa could further confirm of the presence of cirrhosis.

These findings highlight a general variability in the factors regulating the adaptation of the hepatic microcirculation to postprandial hyperemia and, in turn, the changes in liver stiffness. Therefore, not surprisingly, changes in LS values occurring after the meal test do not offer any advantage for discriminating Child A cirrhosis patients from Child B and for predicting the presence or absence of esophageal varices when compared with baseline stiffness values.

In conclusion, the results of the present study provide definitive evidence of the confounding effect of a meal on the accuracy of LS measurements and suggest that a fasting period of 120 minutes should be observed before the performance of TE. The impact of the meal on LS values is proportional to the stage of fibrosis, with the highest delta values in patients with cirrhosis. In this specific stage of the disease, a peak delta LS increase $\geq$8 kPa further confirms the presence of cirrhosis, although, due to a broad individual variability the postmeal variation in LS, do not offer additional diagnostic advantages when compared to basal LS values.

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