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Insulin resistance is a major determinant of liver stiffness in nondiabetic patients with HCV genotype 1 chronic hepatitis

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SUMMARY

Background
In patients with chronic hepatitis C (CHC), liver stiffness measurement (LSM) by transient elastography (TE), is closely related to the stage of fibrosis, but may be affected by necroinflammation. Other factors, such as insulin resistance (IR), might influence the performance of LSM.

Aims
To evaluate in a cohort of nondiabetic patients with genotype 1 CHC, whether IR and other anthropometric, biochemical, metabolic and histological factors contribute to LSM and to identify the best cut-off values of LSM for predicting different stages of fibrosis.

Methods
Nondiabetic patients with genotype 1 CHC (n = 156) were evaluated by liver biopsy (Metavir score), anthropometric, biochemical and metabolic features including IR. Furthermore, all subjects underwent LSM by TE.

Results
Severe fibrosis (F3–F4) was associated with LSM (OR 1.291; 95%CI 1.106–1.508). LSM was also independently correlated with low platelets (P = 0.03), high γGT (P < 0.001) and high HOMA (P = 0.004) levels. A stiffness value ≥8 KPa was identified as the best cut-off for predicting severe fibrosis (AUC 0.870); yet this cut-off still failed to rule out F3–F4 fibrosis in 22.7% of patients (false-negative rate) or rule in F3–F4 in 19.6% (false-positive rate). Platelets <200 × 10^3/mmc and a HOMA of >2.7 were the major determinants of these diagnostic errors in predicting severe fibrosis.

Conclusions
In nondiabetic patients with genotype 1 CHC, insulin resistance, γGT and platelet levels contribute to LSM independently of liver fibrosis. The identification of these three factors contributes to a more correct interpretation of LSM.

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INTRODUCTION

The prognosis of patients with chronic hepatitis C (CHC) is eventually decided by the amount of liver fibrosis.[1] The presence of severe fibrosis (F3–F4) or cirrhosis (F4) represents a state of high risk for the development of liver-related complications. Biopsy, even if invasive, painful and with potentially life-threatening complications, remains the gold standard for the evaluation of liver fibrosis.[2, 3] However, in the past few years, an increasing number of studies have proposed liver stiffness measurement (LSM) by transient elastography (TE) as a rapid, low cost, accurate and non-invasive technique designed to predict the stage of fibrosis and portal hypertension.[4–12]

A recent meta-analysis[12] confirmed the excellent diagnostic performance of LSM in discriminating cirrhosis from all other stages of fibrosis, with a progressive reduction in its diagnostic accuracy when severe fibrosis (F3–F4) or significant fibrosis (F2–F4) were to be distinguished from the milder stages. The meta-analysis[12] identified in the different staging systems, in the country variations and in the different aetiology of the underlying chronic liver disease (HCV-related vs. HCV-unrelated) the major factors of the poor performance of LSM in the diagnosis of significant fibrosis. The study also highlighted the lack of worldwide standardized cut-off values for different stages of fibrosis.

Recent studies have shown that factors other than fibrosis influence liver stiffness values. In fact, even if the accumulation of the fibrillar extracellular matrix and the presence of fibrotic scar tissue are a major determinant of liver tissue stiffness, hepatic necroinflammation, evaluated both by histology and ALT, contributes substantially to liver stiffness.[8–11]

Extrapolating from these data, it is possible to suggest that other, unidentified, factors may influence LSM. Insulin resistance (IR), a feature exceedingly common in patients with CHC,[13–16] particularly in genotype 1 infection,[16] can be considered a putative candidate for influencing LSM variations. In this setting, IR has been systematically associated not only with liver steatosis but also with severe fibrosis,[14, 16] portal hypertension[17, 18] and, ultimately, with the mechanisms leading to collagen deposition, vasoconstriction and regulation of sinusoidal structure.[19–22] Therefore, insulin, interfering with all the above cited mechanisms, could influence liver stiffness.

The aims of this study were to evaluate, in a cohort of nondiabetic patients with genotype 1 CHC, whether IR and other anthropometric, biochemical, metabolic and histological factors contribute to LSM and to identify the best cut-off values of LSM for predicting different stages of fibrosis.

MATERIALS AND METHODS

Patients

The study assessed 156 consecutive patients with G1-CHC recruited at the GI & Liver Unit of the University Hospital in Palermo and fulfilling all inclusion and exclusion criteria detailed below. Patients were included if they had a histological diagnosis of G1 CHC on a liver biopsy performed within 6 months prior to enrolment. G1 CHC patients were characterized by the presence of anti-HCV and HCV RNA, with persistently abnormal alanine aminotransferase (ALT) and liver histology of chronic hepatitis (any degree of fibrosis, including cirrhosis) and by alcohol consumption of <20 g/day in the last year or more, evaluated by questionnaire. Exclusion criteria were: (1) advanced cirrhosis (Child-Pugh B and C); (2) hepatocellular carcinoma; (3) other causes of liver disease or mixed aetiologies (excessive alcohol consumption, hepatitis B, autoimmune liver disease, Wilson's disease, hemochromatosis, α1-antitrypsin deficiency); (4) HIV infection; (5) previous treatment with anti-viral therapy, immunosuppressive drug and/or regular use of steatosis-inducing drugs (corticosteroid, valproic acid, tamoxifen, amiodarone); (6) previous diagnosis of type 1 or type 2 diabetes mellitus, and/or fasting blood glucose ≥126 mg/dL and (7) active IV drug addiction.

The study was performed in accordance with the principles of the Declaration of Helsinki and its appendices, and with local and national laws. Approval was obtained from the hospital's Internal Review Board and Ethics Committee and written informed consent was obtained from all patients.

Clinical and laboratory assessment

Clinical and anthropometric data were collected at the time of liver biopsy. BMI was calculated on the basis of weight in kilograms and height (in meters) and subjects were classified as normal weight (BMI, 18.5–24.9 kg/m^2), overweight (BMI, 25–29.9), obese

(BMI ≥30). Waist circumference was measured at the midpoint between the lower border of the rib cage and the iliac crest. The diagnosis of arterial hypertension was based on the following criteria: systolic blood pressure ≥135 mm Hg and/or diastolic blood pressure ≥85 mm Hg (measured three times within 30 min, in the sitting position and using a brachial sphygmomanometer) or ongoing antihypertensive pharmacological treatment after a diagnosis of arterial hypertension. Metabolic syndrome was diagnosed according to ATPIII criteria.[23] A 12-hr overnight fasting blood sample was drawn at the time of biopsy to determine serum levels of ALT, γ-glutamyltransferase (γ-GT), total cholesterol, HDL and LDL-cholesterol, triglycerides, ferritin, plasma glucose concentration and platelet count. Serum insulin was determined by a two-site enzyme ELISA (Mercodia Insulin ELISA, Arnika). IR was determined by the homeostasis model assessment (HOMA) method, using the following equation:[24] Insulin resistance (HOMA-IR) = Fasting insulin (μU/mL) × Fasting glucose (mmol/L)/22.5. HOMA-IR has been validated in comparison with the euglycemic/hyperinsulinemic clamp technique in both diabetic and nondiabetic subjects.[25] HOMA-IR values of >2.7 were considered an indication of IR; this cut-off corresponds to the upper quartile of a previously published Italian control population.[26] All patients were tested at the time of biopsy for HCV-RNA by qualitative PCR (Cobas Amplicor HCV Test version 2.0; limit of detection: 50 IU/mL). HCV RNA positive samples were quantified by Versant HCV RNA 3.0 bDNA (Bayer Co., Tarrytown, NY, USA) expressed in IU/mL. Genotyping was performed by INNO-LiPA, HCV II, Bayer.

Histology

Slides were coded and read by one pathologist (D.C.) unaware of the patient's identity and history. A minimum length of 15 mm of biopsy specimen or the presence of at least 10 complete portal tracts was required.[27] Biopsies were classified according to the Metavir numerical scoring system.[28] The percentage of hepatocytes containing macrovescicular fat was determined for each 10× field. An average percentage of steatosis was then determined for the entire specimen. Steatosis was assessed as the percentage of hepatocytes containing fat droplets (minimum 5%) and evaluated as a continuous variable. Steatosis was classified as: mild 5–30% or moderate-severe ≥30%.

Liver stiffness measurement

TE was performed using a FibroScan apparatus (Echosens, Paris, France) to measure LSM. The only operator was a staff physician (F.B.) who had previously performed at least 100 determinations in patients with chronic liver disease. The median value of 10 successful acquisitions, expressed in kilopascal (KPa), was maintained as representative of LSM. As previously described in the literature[4] and as suggested by the manufacturing company, we considered 10 successful acquisitions with a success rate of at least 60%, and with an interquartile range lower than 20%, as representative measurements.

Statistics

Continuous variables were summarized as mean ± s.d. and categorical variables as frequency and percentage. The Student's *t*-test was used, when appropriate. Multiple logistic regression models were used to assess the relationship of fibrosis with demographic, virological, metabolic, instrumental and histological characteristics of patients. In this model, the dependent variables were significant fibrosis, coded as 0 = F1 in the fibrosis score or 1 = F2 to F4 in the fibrosis score; severe fibrosis coded as 0 = F1 to F2 in the fibrosis score or 1 = F3 to F4 in the fibrosis score; cirrhosis coded as 0 = F1 to F3 in the fibrosis score or 1 = F4 in the fibrosis score. As candidate risk factors, we selected age, gender, BMI, waist circumference, baseline ALT, platelet count levels, γ-GT levels, ferritin, total-cholesterol, HDL and LDL cholesterol, triglycerides, blood glucose, insulin, HOMA score, arterial hypertension, metabolic syndrome, HCV-RNA levels, steatosis, activity score and LSM.

Multiple linear regression analysis was performed to identify independent predictors of stiffness as a continuous dependent variable. As candidate risk factors for LSM, we selected the same independent variables included in the fibrosis model and added fibrosis as an additional independent variable. Receiver operating characteristic (ROC) curves were applied to find the best cut-off values and to identify the area under ROC curve (*AUC*) of the LSM able to discriminate the different classes of fibrosis. Finally, multiple logistic regression models for LSM, using the best LSM cut-offs for discriminating significant fibrosis, severe fibrosis and cirrhosis as the dependent variables were performed.

Table 1. Demographic, laboratory, metabolic and histological features of 156 nondiabetic patients with genotype 1 chronic hepatitis C

Variable	Chronic hepatitis C genotype 1 (n = 156)
Mean Age – years	52.5 ± 12.5
Gender	
Male	85 (54.5)
Female	71 (45.5)
Mean Body Mass Index – kg/m^2	26.0 ± 3.7
Body Mass Index – kg/m^2	
<25	57 (36.5)
25–29.9	78 (50.0)
≥30	21 (13.5)
Waist circumference- cm	90.2 ± 11.4
Arterial hypertension	
Absent	126 (80.8)
Present	30 (19.2)
Metabolic syndrome (ATPIII)	
Absent	148 (94.9)
Present	8 (5.1)
Alanine aminotransferase – IU	90.8 ± 81.4
Platelet count × 10^3/mmc	205.9 ± 65.3
γ glutamyl transferase – IU	64.5 ± 68.5
Ferritin –ng/mL	254.2 ± 286.7
Cholesterol – mg/dL	174.7 ± 38.8
HDL Cholesterol – mg/dL	54.7 ± 17.7
LDL Cholesterol – mg/dL	105.4 ± 37.0
Triglycerides – mg/dL	97.4 ± 45.0
Blood glucose – mg/dL	87.1 ± 17.5
Insulin – μU/mL	12.39 ± 8.29
HOMA-score	2.64 ± 1.50
Insulin-resistance	
Patients without insulin resistance (HOMA ≤2.7)	89 (57.1)
Patients with insulin resistance (HOMA >2.7)	67 (42.9)
Serum HCV RNA – IU/mL	1044517 ± 917374
Liver Stffness – Kpa	9.0 ± 5.8
Histology at biopsy	
Steatosis:	10.9 ± 17.2
Percent of steatotic hepatocytes	
Categorical variable	
<5%	79 (50.6)
≥5% to <30%	57 (36.6)
≥30%	20 (12.8)
Stage of fibrosis	
1	36 (23.1)
2	76 (48.7)
3	26 (16.7)
4	18 (11.5)
Grade of activity	
1	28 (17.9)
2	95 (60.9)
3	33 (21.2)

IU, international units; HOMA, homeostasis model assessment; HDL, high density lipoprotein; LDL, low density lipoprotein; HCV-RNA, hepatitis C virus ribonucleic acid.

Variables found to be associated with the dependent variable at univariate analyses (probability threshold, $P \leq 0.10$) were included in all multivariate regression models. To avoid the effect of co-linearity, HOMA-score, blood glucose levels and insulin levels, as well as waist circumference and BMI, were not included in the same multivariate model. So, among these variables, we included in the final multivariate model, only the variable with the lower significant P value.

Regression analyses were performed using PROC LOGISTIC, PROC REG and subroutine in SAS (SAS Institute, Inc., Cary, NC, USA).[29]

RESULTS

Patient features and histology

Baseline features of all 156 patients are shown in Table 1. The mean BMI was 26.0 ± 3.7 kg/m^2. Overall, 57 of 156 patients (36.5%) had normal weight, 78 of 156 (50%) were overweight, 21 of 156 (13.5%) were obese. The mean value of HOMA was not especially high (2.64), but 67 of the 156 patients (42.9%) were insulin-resistant. Twenty-eight percent of patients (44/156) had liver fibrosis ≥3 by Metavir score. The proportion of patients with moderate/severe necroinflammation (grading 2–3) was high (72.1%). Histological evidence of steatosis was present in 77 patients (49.4%).

Risk factors for fibrosis

Univariate and multivariate analyses were performed to identify potential association between each feature and fibrosis at three cut-off levels: (a) significant fibrosis (F2–F4); (b) severe fibrosis (F3–F4) and (c) cirrhosis (F4).

Significant fibrosis (F2–F4) was associated with age, triglycerides, cholesterol, ferritin, HOMA, stiffness and necroinflammatory activity. By multivariate analysis, higher triglyceride levels (OR 0.987; 95%CI 0.975–0.999; $P = 0.03$), higher LSM (OR 1.259; 95%CI 1.004–1.578; $P = 0.04$) and high necroinflammatory activity (OR 3.308; 95%CI 1.363–8.032; $P = 0.008$) were independently linked to significant fibrosis.

Age, platelet count, γ-GT, ALT, ferritin, cholesterol, HOMA-IR, LSM, steatosis and necroinflammatory-activity were all linked to severe liver fibrosis (Table 2). Multivariate logistic regression analysis showed that only the following factors were independently associated with severe fibrosis: high necroinflammatory activity (OR 3.784; 95%CI 1.408–10.174; $P = 0.009$), high ferritin levels (OR 1.003; 95%CI 1.001–1.006; $P = 0.04$) and high stiffness values (OR 1.291; 95%CI 1.106–1.508; $P = 0.001$) (Table 2).

Excluding steatosis and grading from the model, as they could be verified only by biopsy, a model was obtained that included age (OR 1.048; 95%CI 1.001–1.097; $P = 0.04$), stiffness values (OR 1.353; 95%CI 1.167–1.569; $P < 0.0001$) and ferritin (OR 1.003; 95%CI 1.001–1.006; $P = 0.04$) as indirect predictors of severe fibrosis. The overall *AUC* of this model was excellent (*AUC* 0.900).

Cirrhosis was associated with age, platelets, γ-GT, ferritin, HOMA, liver stiffness, steatosis and necroinflammatory activity by univariate analysis, but only older age (OR 1.146; 95%CI 1.005–1.306; $P = 0.04$), higher ferritin levels (OR 1.005; 95%CI 1.000–1.011; $P = 0.03$) and higher liver stiffness values (OR 1.433;

Table 2. Univariate and multivariate analysis of risk factors associated with severe fibrosis (F3-F4) in 156 nondiabetic patients with genotype 1 chronic hepatitis C

Variable	No severe fibrosis (Metavir score 1–2) $n = 111$	Severe fibrosis (Metavir score 3–4) $n = 44$	Univariate analysis P value	Multivariate analysis OR (95% CI)	Multivariate analysis P value
Age – years	50.5 ± 12.9	58.1 ± 9.3	0.001	1.045 (0.996–1.097)	0.07
Gender					
Male vs. Female	61/50	23/21	0.76	-	
Body mass index – kg/m^2	26.0 ± 3.8	26.1 ± 3.6	0.91	-	
Waist Circumference- cm	89.4 ± 11.5	92.2 ± 10.9	0.22	-	
Alanine aminotransferase – IU	82.5 ± 79.3	113.7 ± 84.2	0.03	1.002 (0.995–1.008)	0.65
Platelet count × 10^3/mmc	215.7 ± 59.1	180.7 ± 74.4	0.002	0.995 (0.988–1.002)	0.91
γ glutamyl transferase – IU	54.6 ± 53.1	90.3 ± 93.6	0.003	0.995 (0.988–1.002)	0.16
Ferritin –ng/mL	187.9 ± 158.3	425.7 ± 437.2	<0.001	1.003 (1.001–1.006)	0.04
Cholesterol – mg/Dl	179.1 ± 36.0	163.1 ± 43.8	0.02	0.986 (0.970–1.002)	0.09
HDL Cholesterol – mg/Dl	55.8 ± 18.2	51.9 ± 16.3	0.24	-	
LDL Cholesterol – mg/Dl	108.5 ± 33.2	96.9 ± 45.2	0.12	-	
Triglycerides – mg/Dl	99.7 ± 48.4	93.1 ± 34.2	0.41	-	
Blood glucose – mg/dL	85.5 ± 18.5	91.1 ± 14.5	0.07	-	
Insulin – μU/mL	12.0 ± 9.0	13.5 ± 5.8	0.30	-	
HOMA-score	2.49 ± 1.51	3.06 ± 1.39	0.03	0.748 (0.484–1.157)	0.19
Arterial hypertension					
Absent vs. present	89/22	36/8	0.81	-	
Metabolic syndrome (ATPIII)					
Absent vs. present	105/6	42/2	0.82	-	
HCV-RNA- IU	1144302 ± 955089	445810 ± 264472	0.33	-	
Liver Stiffness – KPa	6.8 ± 3.1	14.5 ± 7.3	<0.001	1.291 (1.106–1.508)	0.001
Histology at biopsy					
Steatosis	8.9 ± 15.0	16.0 ± 21.1	0.02	0.997 (0.964–1.031)	0.85
Grade of inflammation	1.8 ± 0.5	2.4 ± 0.5	<0.001	3.784 (1.408–10.174)	0.009

IU, international units; HOMA, homeostasis model assessment; HDL, high density lipoprotein; LDL, low density lipoprotein; HCV-RNA, hepatitis C virus ribonucleic acid.

95%CI 1.143–1.796; $P = 0.002$) were confirmed as independent factors at multivariate analysis.

Factors associated with LSM

The mean LSM value was 9.0 KPa (range 2.7–37.5). Liver stiffness values significantly increased according to fibrosis stage (6.1 ± 1.7 in F1; 7.2 ± 3.5 in F2; 10.5 ± 3.6 in F3; 20.3 ± 7.5 in F4; $P < 0.001$). In the evaluation of factors associated with liver stiffness, multivariate linear regression analyses highlighted that stiffness was independently correlated with stage of fibrosis, low platelet levels, high γ-GT levels and high HOMA-score (Table 3).

ROC curves identified the best cut-offs of liver stiffness at 6.5 KPa for the diagnosis of significant fibrosis (*AUC* 0.697; Sensitivity 61%, Specificity 64%), at 8 KPa for severe fibrosis (*AUC* 0.870; Sensitivity 78%, Specificity 81%) and at 14.5 KPa for cirrhosis (*AUC* 0.954; Sensitivity 83%, Specificity 85%).

Considering the performance of stiffness at the cut-off value of 6.5 KPa in discriminating significant fibrosis as not acceptable, we tried to identify factors associated with stiffness cut-offs for severe fibrosis (8 KPa) and cirrhosis (14.5 KPa). Liver stiffness ≥ 8 KPa was associated with stage of fibrosis (OR 4.330; 95%CI 1.758–10.665; $P = 0.001$), low platelet levels (OR 0.982; 95%CI 0.968–0.997; $P = 0.01$) and higher HOMA score (OR 1.975; 95%CI 1.256–3.106; $P = 0.003$) by multivariate analysis. The ROC curve identified platelet levels of $<200 \times 10^3$/mmc and a HOMA score of >2.7 as the best cut-off values for liver stiffness ≥8 KPa. Figure 1 shows the stiffness distribution within both F1–F2 fibrosis stages and F3–F4 fibrosis stages according to platelet (Figure 1a) and HOMA (Figure 1b) cut-off levels. Figure 2a and 2b show false-positive (19.6%) and false-negative (22.7%) rates, according to platelet and HOMA levels, at stiffness cut-off of 8 KPa for severe fibrosis. Interestingly, the mean of liver stiffness in false-positive and

Table 3. Univariate and multivariate analysis of factors associated with liver stiffness in 156 nondiabetic patients with genotype 1 chronic hepatitis C

	Univariate analysis			Multivariate analysis		
Variable	β	S.E.	*P* value	B	S.E.	*P* value
Mean Age – years	0.289	0.036	<0.001	−0.017	0.026	0.80
Male gender	0.014	0.942	0.85		–	
Mean Body Mass Index – kg/m^2	0.182	0.124	0.02		–	
Waist circumference- cm	0.296	0.039	0.01	0.028	0.029	0.67
Alanine aminotransferase – IU	0.248	0.006	0.002	0.071	0.004	0.32
Platelet count × 10^3/mmc	−0.366	0.007	<0.001	−0.147	0.006	0.03
γ glutamyl transferase – IU	0.450	0.006	<0.001	0.279	0.007	<0.001
Ferritin –ng/mL	0.520	0.001	<0.001	0.026	0.002	0.74
Cholesterol – mg/dL	−0.161	0.012	0.04	0.034	0.008	0.59
HDL Cholesterol – mg/dL	−0.125	0.027	0.13		–	
LDL Cholesterol – mg/dL	−0.026	0.013	0.77		–	
Triglycerides – mg/dL	0.123	0.010	0.12		–	
Blood glucose – mg/dL	0.180	0.026	0.02		–	
Insulin – μU/mL	0.221	0.056	0.006		–	
HOMA-score	0.367	0.294	<0.001	0.202	0.228	0.004
Arterial hypertension	−0.011	1.190	0.89		–	
Metabolic Syndrome (ATPIII)	0.060	2.122	0.45		–	
Serum HCV RNA – IU/mL	0.001	0.000	0.99		–	
Histology at biopsy						
Steatosis	0.190	0.027	0.01	−0.040	0.022	0.54
Stage of fibrosis	0.640	0.377	<0.001	0.509	0.413	<0.001
Grade of activity	0.441	0.674	<0.001	0.033	0.581	0.65

IU, international units; HOMA, homeostasis model assessment; HDL, high density lipoprotein; LDL, low density lipoprotein; HCV-RNA, hepatitis C virus ribonucleic acid.

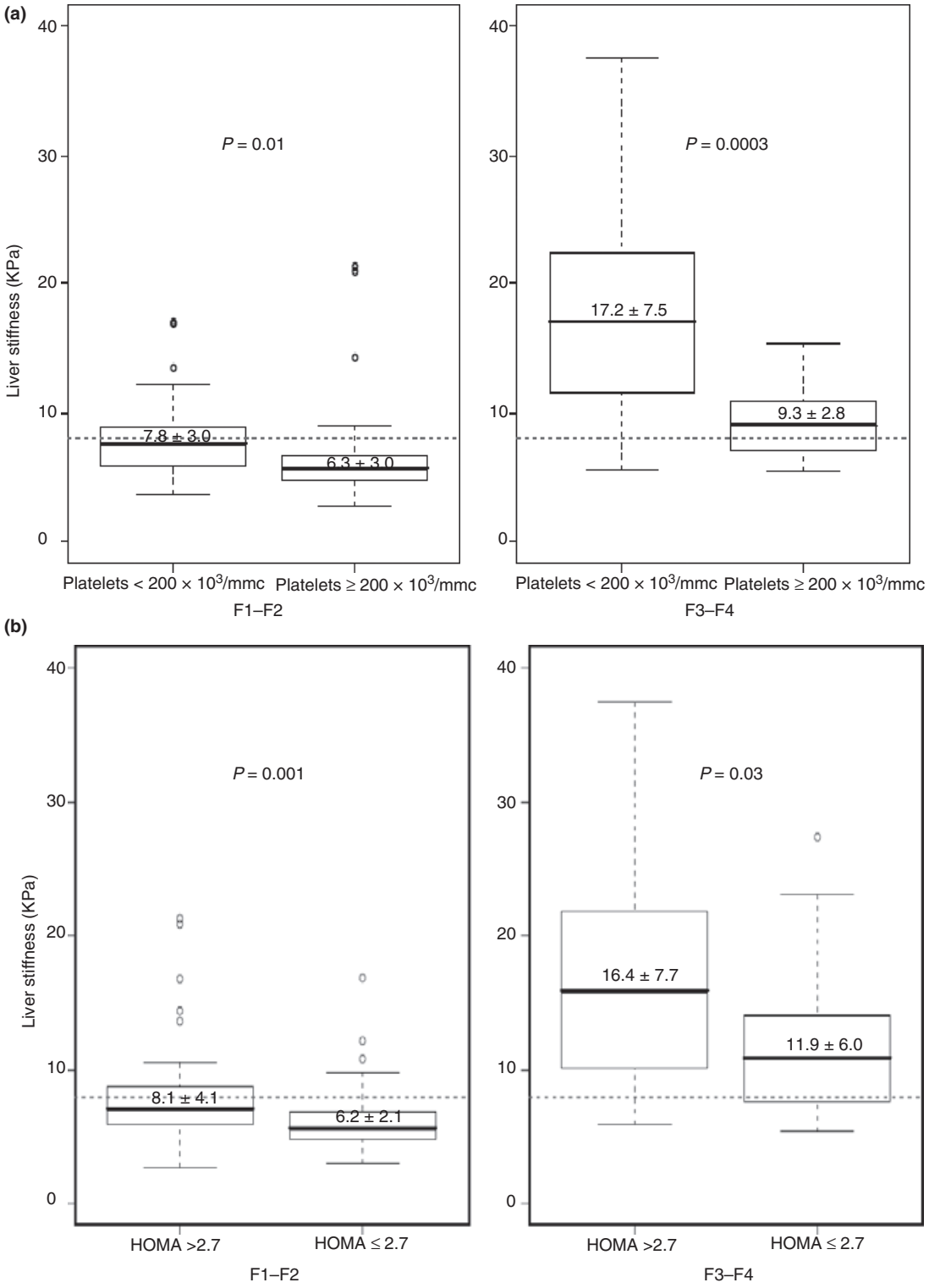

Figure 1. Stiffness values distribution within patients with F1-F2 fibrosis stages and F3-F4 fibrosis stages, according to platelet cut-off of 200 × 10^3/mmc (a) and HOMA cut-off of 2.7 (b). The horizontal bar inside the box represents the media. The upper and lower ends of the box (hinges) represent the approximate upper and lower quartiles of the data distribution. Extreme data are collected to the ends of the box by vertical bars.

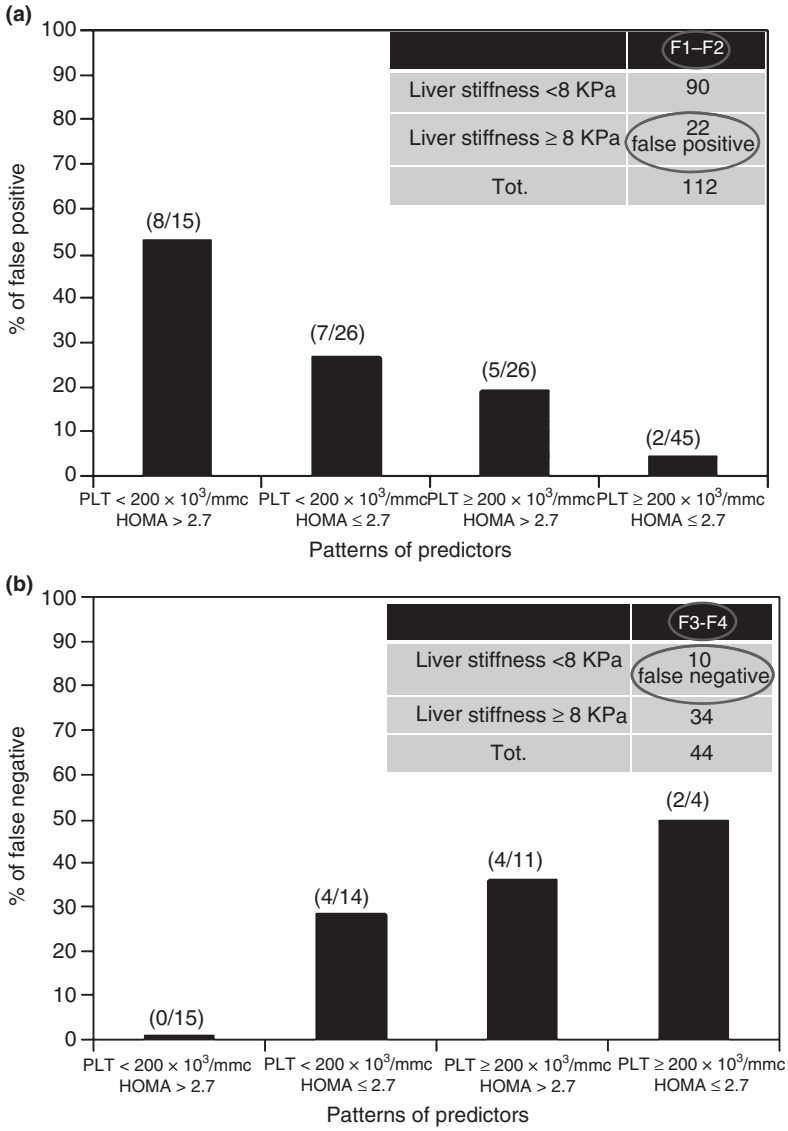

Figure 2. False-positive rate in patients with F1-F2 fibrosis (a) and false-negative rate in patients with F3-F4 fibrosis (b), according to platelet and HOMA cut-off levels. The overall false-positive rate was 19.6% (22/112) and the overall false-negative rate was 22.7% (10/44) at the liver stiffness cut-off of 8 KPa for severe fibrosis. The false-positive rate progressively decreased from patients in the worst class (Platelets<200 × 10^3/mmc and HOMA>2.7) to those in the best class (Platelets≥200 × 10^3/mmc and HOMA≤2.7) (Figure 2A). False-negative rate progressively increased from patients in the best class (Platelets<200 × 10^3/mmc and HOMA>2.7) to those in the worst class (Platelets≥200 × 10^3/mmc and HOMA≤2.7) (Figure 2B).

false-negative patients was 11.6 ± 4 KPa (range 8.3–21.3 KPa) and 6.8 ± 0.9 KPa (range 5.4–7.8 KPa) respectively. Multivariate analysis showed that liver stiffness ≥ 14.5 KPa was associated with stage of fibrosis (OR 9.823; 95%CI 1.725–55.945; $P = 0.01$), low platelet levels (OR 0.969; 95%CI 0.940–0.998; $P = 0.03$) and higher γ-GT levels (OR 1.036; 95%CI 1.004–1.069; $P = 0.02$). ROC curves identified platelets of 165 × 10^3/mmc and γ-GT of 78.5 UI/mL as the best cut-off values for liver stiffness ≥ 14.5 KPa. Interestingly, in the group of cirrhotic patients, we observed a significant difference in stiffness values according to the platelet cut-off of 165 × 10^3/mmc (22.3 ± 6.3 KPa vs. 15.2 ± 8.6 KPa) and to the γ-GT cut-off of 78.5 UI/mL (23.6 ± 6 UI/mL vs. 13.7 ± 5.8 UI/mL). Similar results were obtained considering the group of

patients without cirrhosis (data not shown). Estimating the performance of liver stiffness = 14.5 KPa for cirrhosis, we found a false-positive rate of 5.7% and false-negative rate of 16%, according to platelet and γ-GT levels. The observed mean of LSM was 17.8 $\pm$ 2.1 KPa (range 15.3–21.3 KPa) and 9 $\pm$ 1.1 KPa (range 8–10.2 KPa) in false-positive and false-negative patients respectively.

DISCUSSION

In this study of 156 nondiabetic patients with G1 CHC, we confirmed that the performance of LSM was not acceptable for significant fibrosis, good for severe fibrosis and excellent for cirrhosis.[30] Importantly, we found that not only fibrosis but also other factors, namely platelets, γGT and HOMA levels were closely and independently linked to stiffness values. Each of the latter would thus be able to affect the diagnostic performance of LSM in evaluating the stage of fibrosis.

A novel finding of our work, not specifically evaluated in other studies, is the association of IR with stiffness values. IR has been systematically associated with fibrosis[14, 16] and with portal hypertension[17, 18] in several reports. The association between IR and fibrosis has a consistent biological plausibility, considering the ability of insulin to stimulate hepatic stellate cells[19] and to induce connective growth factor production.[20] A second mechanism by which IR could interfere with LSM is the induction of portal hypertension via modulation of nitric oxide and endothelin synthesis.[21, 22] Therefore, insulin could influence liver stiffness values, even in a pre-cirrhotic state with relatively modest fibrosis, by interfering with both mechanical and dynamic mechanisms, leading to collagen deposition, vasoconstriction and regulation of sinusoidal structure.

Along this line, platelet levels represent an indirect marker of fibrosis severity[14] and of portal hypertension,[17, 31] two factors strongly associated with higher liver stiffness.[4–12]

Recent studies[8–11]have also underscored that liver inflammation, expressed by necroinflammatory hepatic activity or ALT levels, contributes significantly to hepatic stiffness, interfering with the performance of TE in staging fibrosis. In this study, we confirmed the direct relation between stiffness and both liver necroinflammation and ALT levels. However, these associations were not confirmed at multivariate analysis. Differences in the aetiology of liver disease, in the prevalence of alcohol abuse and diabetes and in the different staging scoring systems used, may explain these different results. Furthermore, we found that γGT, but not ALT, was an independent factor influencing LSM. In fact, serum γGT could interfere with LSM not only as a surrogate marker of liver TNF-α levels[32] and therefore of hepatic inflammation but also as a simple and reliable marker of cholestasis,[33] visceral fat,[34] steatosis[13] and fibrosis.[35] Finally, we cannot rule out that other unknown mechanisms could be responsible of the independent association between γGT and liver stiffness.

This study adds further evidence to the lack of association between stiffness values and steatosis.[11]

Liver stiffness measurement, as also confirmed by our study, appears to be a new tool capable of independently predicting the stage of fibrosis in patients with G1 CHC.[4–12] We investigated the performance of LSM in the diagnosis of different stages of fibrosis, identifying LSM values of 6.5 KPa, 8 KPa and 14.5 KPa as the best cut-offs for discriminating significant fibrosis (F2–F4), severe fibrosis (F3–F4) and cirrhosis (F4) respectively. These stiffness cut-off values were different from those identified in other studies. This may be an expression of both the different distribution of stiffness confounders and the different prevalence of fibrosis stages in the various populations. In our group of patients, LSM was not acceptable in discriminating significant fibrosis (*AUC* 0.697), while we confirmed it to be a useful tool for predicting severe fibrosis (*AUC* 0.870) and cirrhosis (*AUC* 0.954), even if influenced by the factors identified above.

Using a cut-off of 8 KPa for severe fibrosis, the false-negative (22.7%) and false-positive (19.6%) rates were relevant and related to platelet and HOMA levels. Similarly, at the cut-off of 14.5 KPa for cirrhosis, false-negative (16%) and false-positive (5.7%) rates were clinically significant and related to platelet and γGT levels.

The study has limitations. First, the analysis was carried out in a relatively small number of patients. So, multivariate analyses, particularly in cirrhotic patients, could be exposed to the risk of a low precision estimate because of a possible model overfitting. Accordingly, it will be interesting to see if these results also hold true in larger groups of patients with CHC and in patients with liver disease of other aetiology. Second, our study included a cohort of European nondrinker patients with low prevalence of obesity and cirrhosis, who were enrolled in a tertiary referral

centre for liver disease, limiting the broad application of the results. A further methodological issue could reside in the accuracy of liver biopsy examination for assessing fibrosis, related to sampling errors, technical processing of the specimens, intra- and inter-observer variability and both to the length of biopsy specimens and the number of portal spaces. However, we are confident that the minimum length of 15 mm and the presence of at least 10 complete portal tracts minimize this bias. Finally, we cannot exclude the possibility that hidden abuse of alcohol may be responsible for the presence of stiffness variations in a few subjects.

In conclusion, we believe that the evidence is sufficient to consider that in patients with G1 CHC, the performance of LSM is not acceptable for significant fibrosis, good for severe fibrosis and excellent for cirrhosis. However, in patients with severe fibrosis and cirrhosis platelet, γGT and HOMA levels could be responsible of the presence of liver stiffness values higher or lower than expected according to their fibrosis stage.

Finally, we feel, we have provided further evidence of a more correct interpretation of LSM, suggesting that LSM alone is a useful, although not sufficient, tool for staging liver fibrosis.

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