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Presence of Diabetes Mellitus and Steatosis Is Associated With Liver Stiffness in a General Population: The Rotterdam Study

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Given that little is known about the prevalence of, and factors associated with, liver fibrosis in the general population, we aimed to investigate this in a large, well-characterized cohort by means of transient elastography (TE). This study was part of the Rotterdam Study, a population-based study among individuals ≥45 years. All participants underwent abdominal ultrasound and TE. Liver stiffness measurement (LSM) ≥8.0 kilopascals (kPa) was used as a cutoff suggesting clinically relevant fibrosis. Of 3,041 participants (age, 66.0 ± 7.6 years) with reliable LSM, 169 (5.6%) participants had LSM ≥8.0 kPa. Age (odds ratio [OR]: 2.40; 95% confidence interval [CI]: 1.72-3.36; $P < 0.001$), alanine aminotransferase (ALT; OR, 1.24; 95% CI: 1.12-1.38; $P < 0.001$), smoking (OR, 1.77; 95% CI: 1.16-2.70; $P = 0.008$), spleen size (OR, 1.23; 95% CI: 1.09-1.40; $P = 0.001$), hepatitis B surface antigen, or anti–hepatitis C virus positivity (OR, 5.38; 95% CI: 1.60-18.0; $P = 0.006$), and combined presence of diabetes mellitus (DM) and steatosis (OR, 5.20; 95% CI: 3.01-8.98; $P < 0.001$ for combined presence) were associated with LSM ≥8.0 kPa in multivariable analyses. The adjusted predicted probability of LSM ≥8.0 kPa increased per age decade, with probabilities ranging from 1.4% (0.9-3.6) in participants ages 50-60 years to 9.9% (6.8-14.5) in participants >80 years. Participants with both DM and steatosis had the highest probabilities of LSM ≥8.0 kPa (overall probability: 17.2% [12.5-23.4]; this probability did not increase with age [$P = 0.8$]). *Conclusion*: In this large population-based study of older adults, LSM ≥8.0 kPa, suggestive of clinically relevant fibrosis, was present in 5.6% and was strongly associated with steatosis and DM. In the context of an aging population and an increased prevalence of DM and obesity, this study illustrates that liver fibrosis may become a more prominent public health issue in the near future. (HEPATOLOGY 2016;63:138-147)

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Advanced liver fibrosis and cirrhosis affects hundreds of millions of people and comprises a major cause of morbidity and mortality, with a world-wide mortality rate owing to liver cirrhosis (LC) and primary liver cancer of over 1.5 million per year.[1,2] In the United States, chronic liver disease (CLD) and cirrhosis are the eleventh leading cause of mortality.[3] In the past few decades, nonalcoholic fatty liver disease (NAFLD) has become one of the major causes of liver disease.[4] In previous

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; CI, confidence interval; CLDs, chronic liver diseases; DM, diabetes mellitus; FPG, fasting plasma glucose; GGT, gamma-glutamyl transferase; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; HTN, hypertension; IQR, interquartile range; kPa, kilopascal; LC, liver cirrhosis; LSM, liver stiffness measurement; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; PPV, positive predictive value; TE, transient elastography; TGs, triglycerides; ULN, upper limit of normal; US, ultrasound; USG, ultrasonography.

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European population-based cohort studies, the reported prevalence of steatosis, assessed by abdominal ultrasound (US), ranged from 25% to 35%.[5-8] In patients with diabetes mellitus (DM), the prevalence of NAFLD was shown to be as high as 40%-70%.[9,10] Considering population aging and the current epidemic in obesity and type II DM in developed countries, the burden of CLDs—in particular, nonalcoholic steatohepatitis (NASH)—is projected to increase substantially in the next decades.[11-13]

To date, limited studies have been performed focusing on the prevalence of, and risk factors for, liver fibrosis in the general population, owing to the fact that data are mainly derived from autopsy studies or biopsy studies in selected populations. In theory, one way to investigate the prevalence of liver fibrosis and cirrhosis in the general population would be to perform a liver biopsy in a large population of healthy volunteers. However, an important disadvantage of liver biopsies is the invasive nature of the procedure, and healthy volunteers are rarely representative of the general population at risk. In recent years, noninvasive techniques have been developed to circumvent the need for liver biopsy. One of these techniques is transient elastography (TE), which is strongly associated with histological stages of liver fibrosis.[14-19] Previous studies examining factors associated with elevated liver stiffness measurements (LSMs) in community-based populations were performed in volunteers attending a free medical checkup and in a Chinese population-based cohort study.[20,21] Until now, these factors have not been studied in a large random sample of a general Caucasian population. Therefore, the aim of our study was to investigate the distribution of, and factors associated with, clinically relevant liver fibrosis, as measured by TE, in a large population-based cohort of Caucasians.

Subjects and Methods

Study Population. The Rotterdam Study is a large, prospective, population-based cohort study conducted among adults age 45 years and above living in Ommoord, a district of Rotterdam, The Netherlands. The rationale and study design have been described previously.[22] The medical ethics committee at the Erasmus University of Rotterdam approved the study, and written informed consent was obtained from all participants.

All consecutive participants (age range: 51-98 years) that visited the research center between January 2011 and September 2013 were included in the current study. Each participant completed an extensive interview and clinical examination that included abdominal ultrasonography (USG), TE, a fasting blood collection, and an anthropometric assessment.

Interview. The interview preceded the clinical examination and was designed to obtain data concerning demographics, medical history, comorbid conditions, current and past smoking behavior, current alcohol consumption (drinks/week), and drug use. Detailed information on dispensed drug prescriptions was obtained from automated pharmacies, where nearly all participants are registered.

Biochemistry. Fasting blood samples were collected on the morning of US examination and LSM. Blood lipids, platelet count, glucose, and alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), and total bilirubin were measured using automatic enzymatic procedures (Roche Diagnostics GmbH, Mannheim, Germany). Insulin, hepatitis B surface antigen (HBsAg), and anti-hepatitis C virus (anti-HCV) were measured by an automatic immunoassay (Roche Diagnostics GmbH). According to local cut-off criteria, the upper limit of normal (ULN) of ALT was defined as 40 U/L for men and 30 U/L for women.

Abdominal USG. Abdominal USG was performed by a certified and experienced technician on a Hitachi HI VISION 900 (Hitachi Medical Corporation, Tokyo, Japan). Images were stored digitally and reevaluated by a single hepatologist with more than 10 years of experience in USG (J.S.). Diagnosis of steatosis was determined by the US technician according to the protocol by Hamaguchi et al.[23] Severity of fatty liver was

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classified as no fatty liver (score 0-1), mild fatty liver (score 2-3), or moderate-to-severe fatty liver (score 4-6). NAFLD was defined as steatosis in the absence of any of the following possible secondary causes of steatosis: (1) excessive alcohol consumption (>14 drinks/week); (2) positive HBsAg or anti-HCV tests; and (3) use of pharmacological agents associated with steatosis (i.e., amiodarone, corticosteroids, methotrexate, and tamoxifen). In addition, spleen size, presence of collaterals, and Doppler examination of hepatic veins, hepatic artery, and portal vein were evaluated during US examination. Splenomegaly was defined as a spleen size >12.0 cm.

TE. LSM (FibroScan; Echosens, Paris, France) was performed by a single operator, who carried out more than 1,000 examinations before the start of the study. LSM was performed on the right lobe of the liver, through the intercostal spaces, with the participant lying flat on his or her back with the right arm laying in maximal abduction. Either M- or XL-probe was applied, according to instructions by the manufacturer. Participants with intracardiac devices and participants with physical disabilities, making TE impossible, were excluded from the study. Because of necessary maintenance of probes (for calibration), TE was not performed on 4 days during the study period. Failure was recorded when no LSM was obtained after at least 10 shots. Reliability of LSM was categorized according to the criteria by Boursier et al.[24] LSM was considered poorly reliable if interquartile range (IQR)/median LSM >0.30 with median LSM ≥7.1 kilopascals (kPa). These poorly reliable LSMs were excluded from our analyses. LSM ≥8.0 kPa and >13.0 kPa were taken as cutoffs suggesting clinically relevant liver fibrosis and cirrhosis, respectively. These cut-off levels were deliberately chosen because they are known to yield high positive predictive values (PPVs) for presence of clinically relevant fibrosis and cirrhosis in previous studies.[16,21,25]

Covariables. Anthropometric measurements were performed by well-trained research assistants. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Waist circumference was measured in centimeters. The average of two blood pressure (BP) measurements, obtained at a single visit in the upright position after a minimum of 5 minutes of rest, was used for analysis. Metabolic syndrome (MetS) was defined, according to Adult Treatment Panel III criteria,[26] as the presence of at least three of the following five traits: (1) abdominal obesity, defined as a waist circumference in men >102 cm (40 inches) and in women >88 cm (35 inches); (2) serum triglycerides (TGs) ≥150 mg/dL (1.7 mmol/L) or drug treatment for elevated TGs; (3) serum HDL cholesterol (HDL-C) <40 mg/dL (1.0 mmol/L) in men and <50 mg/dL (1.3 mmol/L) in women or drug treatment for low HDL-C; (4) BP ≥130/85 mmHg or drug treatment for elevated BP; and (5) fasting plasma glucose (FPG) ≥100 mg/dL (5.6 mmol/L) or drug treatment for elevated blood glucose. Hypertension (HTN) was defined as BP ≥140/90 mmHg or drug treatment for elevated BP. DM was defined as FPG ≥126 mg/dL (7.0 mmol/L) or drug treatment for elevated blood glucose. The insulin resistance index was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR): fasting glucose (mmol/L) × fasting insulin (mU/L)/22.5 [27].

Statistical Analysis. Baseline data were done using descriptive statistics. Chi-square tests and Student *t* tests (means) or Wilcoxon's rank-sum tests (medians) were used to assess the significance of differences in distributions of categorical data and continuous data, respectively. To examine associations between traits and LSM as continuous (log-transformed) dependent variable or LSM ≥8.0 kPa, we performed linear or logistic regression analyses, respectively. Given that there is some discord regarding the cut-off level of LSM for presence of advanced liver fibrosis using XL-probe, we performed additional sensitivity analyses and tested our model applying a 1 point lower cutoff for XL probe.[15] In addition, we tested our model applying a cutoff of 9.5kPa for advanced fibrosis, a cutoff that has been proposed in studies including subjects with viral hepatitis.[25] Interaction terms between age, sex, and other covariables were tested in linear and logistic multivariable regression models. A combined interaction term of DM and steatosis with age was constructed by dividing the total cohort into four groups: (1) participants without DM and steatosis; (2) participants with DM and without steatosis; (3) participants with steatosis and without DM; and (4) those with both DM and steatosis. We calculated predicted probabilities of having LSM ≥8.0 kPa by using all covariables of our multivariable logistic regression model (i.e., age, sex, spleen size, ALT, BMI, alcohol consumption/week, smoking behavior, presence of HBsAg and/or anti-HCV, presence of DM and/or steatosis, and the combined interaction term). Probabilities were expressed as median (IQR) percentage. Linear regression analyses were used to assess the significance of differences in predicted probabilities between subgroups of our cohort. A P value of <0.05 was considered statistically significant. Statistical analyses were performed using SPSS software (version 21.0; SPSS, Inc., Chicago, IL).

Results

Study Population. Of 3,439 participants age 51 or above who visited the research center between January 2011 and September 2013, a total of 3,342 underwent TE (Fig. 1). Because of LSM failure, an additional 162 participants (4.8%) were excluded. LSM was reliable in 3,041 participants. Baseline characteristics of the study population are shown in Table 1. Fifty-five percent of 3,041 participants were women, mean age of participants was 66.0 ± 7.6 years, and mean BMI was 27.3 ± 4.0 kg/m^2. Participants were predominantly of Caucasian ethnicity (95.1%). Median LSM was 4.7 kPa (3.8-5.8) and 1,080 participants (35.5%) had presence of steatosis on abdominal US. LSM ≥8.0 kPa, suggesting presence of clinically relevant fibrosis, was detected in 169 participants (5.6%). Twenty-two participants had positive viral serology (7 were HBsAg positive and 16 were anti-HCV positive; 1 participant was both HBsAg and anti-HCV positive); only 1 of these participants had ALT of 70U/L, which is 1× the ULN. Normal liver enzymes were present in 2,216 (76.0%) of all participants. Of these 2,216 participants, 81 (3.7%) had LSM ≥8.0 kPa.

Factors Associated With Liver Stiffness. Distribution of LSM values in our cohort of 3,041 participants is illustrated in Fig. 2. Results of linear univariable analyses are illustrated in Supporting Table 1. In linear multivariable regression analysis, log-transformed LSM was associated with higher age, male sex, presence of DM, larger spleen size, higher ALT, presence of positive viral serology for hepatitis B and/or C, and presence of steatosis (Table 2). Greater HOMA-IR, indicative of more insulin resistance, was associated with higher LSM when we replaced presence of DM with HOMA-IR in the model ($\beta = 0.003$; 95% confidence interval [CI]: 0.001-0.004; $P < 0.001$). Replacing BMI with waist circumference in the model did not alter the association with LSM ($\beta = -0.001$; 95% CI: −0.001 to <0.001; $P = 0.08$). No interaction between age, sex, and other covariables was observed for the association with LSM.

Using linear regression analysis in 1,565 participants without apparent liver disease, excluding participants with positive HBsAg, anti-HCV, steatosis, excessive alcohol intake, and ALT above the ULN, only higher age ($\beta = 0.029$ per 10 years; 95% CI: 0.020-0.037; $P < 0.001$) and male sex ($\beta = 0.066$; 95% CI: 0.049-0.082; $P < 0.001$) remained associated with log LSM. In these participants, the 5th and 95th percentile values of LSM were 2.8 and 7.3 kPa, respectively. Age ($\beta = 0.021$ per 10 years; 95% CI: 0.013-0.030; $P < 0.001$) and male sex ($\beta = 0.067$; 95% CI: 0.051-0.082; $P < 0.001$) remained associated with higher log LSM after excluding an additional 51 cases with LSM ≥8.0 kPa.

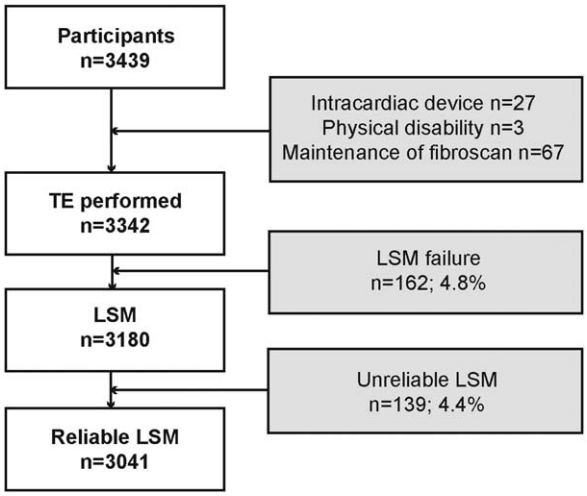

Fig. 1. Flowchart of the study. In total, 3,041 participants had a reliable liver stiffness measurement.

Factors Associated With and Predicted Probability of Clinically Relevant Fibrosis. Of 3,041 participants, 169 (5.6%) had LSM ≥8.0 kPa, suggesting clinically relevant fibrosis, and 19 (0.6%) had LSM >13.0 kPa, suggesting presence of advanced fibrosis or cirrhosis. General characteristics of participants according to an LSM cutoff of 8.0 kPa are shown in Table 1. One participant with LSM ≥8.0 kPa had positive serum anti-HCV, and 3 had positive serum HBsAg. Results of logistic univariable analyses are illustrated in Supporting Table 2. Factors associated with LSM ≥8.0 kPa in multivariable logistic regression analysis are shown in Table 3. Severity of steatosis was not associated with elevated LSM, applying different cutoffs ($P = 0.31$ for ≥8.0 kPa and $P = 0.79$ for ≥9.5 kPa). Thirteen of nineteen (68%) participants with LSM >13.0 kPa were female; mean age of this group was 70.0 ± 11.8 years and mean BMI 28.7 ± 6.4 kg/m^2. Two of these participants drank more than 14 alcoholic beverages per week, 11 (57.9%) participants had steatosis on USG, and 7 (38.9%) had DM. Splenomegaly was present in 5 of 19 participants with LSM >13.0 kPa. None of the participants with LSM >13.0 kPa had positive viral serology for hepatitis B or C.

We performed several sensitivity analyses to determine the robustness of our findings. Consistency was shown, when a 1 point lower cutoff for elevated LSM was applied for use of the XL probe or when 9.5 kPa was used as a cutoff for clinically relevant liver fibrosis. When participants with ALT >2× the ULN were excluded from the analyses, given that severe

Table 1. Baseline Characteristics

	Total	<8.0 kPa	≥8.0 kPa	*P* Value*
Characteristic	**n = 3,041**	**n = 2,872**	**n = 169 (5.6%)**	
Age, years	66.0 ± 7.6	65.8 ± 7.5	68.7 ± 8.9	<0.001
Female	55.0	56.1	37.9	<0.001
Caucasian	95.1	95.0	96.9	0.3
BMI, kg/m^2	27.3 ± 4.0	27.2 ± 3.9	28.9 ± 5.2	<0.001
Normal; BMI <25	28.5	28.9	22.2	
Overweight; 25 ≤ BMI < 30	49.2	49.7	40.1	
Obese; BMI ≥30	22.3	21.4	37.7	
Waist circumference, cm	93.7 ± 12.2	93.3 ± 12.0	99.7 ± 14.2	<0.001
Alcohol consumption, drinks/week	3.8 (0.4-8.3)	3.8 (0.4-8.3)	3.8 (0.4-8.8)	0.4
Presence of HBsAg and/or anti-HCV	0.8	0.7	2.5	0.03
Smoking				0.002
Never	35.1	35.9	22.2	
Former	51.8	51.2	61.1	
Current	13.1	12.9	16.7	
HTN†	62.4	61.5	76.7	<0.001
DM‡	11.1	9.8	33.7	<0.001
MetS	44.0	42.9	61.9	<0.001
Waist circumference >88 cm (♀) or >102 cm (♂)	44.6	43.9	56.9	0.001
TGs >150 mg/dL or drug treatment for elevated TGs	37.2	36.8	44.3	0.05
HDL-C <40 mg/dL(♂) or <50 mg/dL(♀) or drug treatment for low HDL-C	33.5	33.0	42.5	0.01
BP ≥130/85 mmHg or drug treatment for elevated BP†	77.7	77.2	86.0	0.01
FPG >100 mg/dL or drug treatment for elevated blood glucose	44.7	43.2	69.9	<0.001
US characteristics				
Steatosis	35.5	34.1	59.2	<0.001
Spleen size, cm§	9.8 ± 1.3	9.8 ± 1.3	10.4 ± 1.5	<0.001
Portal vein flow velocity, cm/s‖	22.0 ± 4.7	22.0 ± 4.7	22.2 ± 5.4	0.6
Laboratory data				
ALT, U/L	18 (14-24)	18 (14-24)	22 (16-30)	<0.001
AST, U/L	24 (21-28)	24 (21-28)	27 (23-36)	<0.001
Bilirubin, umol/L	8 (6-11)	8 (6-11)	9 (6-12)	0.07
ALP, U/L	67 (56-78)	67 (56-78)	70 (56-87)	0.08
GGT, U/L	24 (17-35)	24 (17-34)	38 (25-67)	<0.001
HOMA-IR	2.6 (1.7-3.9)	2.5 (1.7-3.8)	3.9 (2.5-6.3)	<0.001
Platelets,*10^9/L	263 (224-306)	263 (226-307)	242 (205-288)	<0.001
TE				
LSM (kPa)	4.7 (3.8-5.8)	4.6 (3.8-5.6)	9.1 (8.6-10.4)	<0.001
IQR/M	0.19 (0.12-0.27)	0.19 (0.12-0.27)	0.17 (0.12-0.24)	0.02

Data are represented as mean ± standard deviation, median (25th-75th percentile), or percentage.

*Based on *t* test, Wilcoxon's rank-sum test, chi-square test, or Fisher's exact test.

†BP measurement and data on drug treatment for elevated BP were available for 2,611 participants.

‡Data on presence of DM were obtained in 3,001 participants.

§Spleen-size measurement was available for 2,587 participants.

‖Portal blood flow velocity was available for 2,887 participants.

Abbreviation: IQR/M, interquartile range/median LSM.

inflammation of the liver may have influenced the PPV of presence of liver fibrosis, the prevalence of LSM ≥8.0 kPa was 5.2% and the same factors, with sex instead of spleen size, remained associated with elevated log-transformed LSM.

We calculated predicted probabilities of having LSM ≥8.0 kPa, taking into account all factors associated with LSM ≥8.0 kPa in our multivariable model. These predicted probabilities increased incrementally with age (Fig. 3). The adjusted probability of having LSM ≥8.0 kPa was 1.4% (0.9-3.6) for participants of 50-60 years of age, 3.4% (2.1-6.0) for those ages 60-70 years, 5.5% (3.6-8.5) for participants ages 70-80 years, and 9.9% (6.8-14.5) for participants >80 years.

Given that the association between age and LSM ≥8.0 kPa was significantly influenced by the presence of DM and/or steatosis (*P* for combined interaction term = 0.04), we also calculated predicted probabilities of having LSM ≥8.0 kPa per age decade for participants with and without DM and/or steatosis (Fig. 4). These probabilities increased per age decade for participants without DM or steatosis (n = 1,814; 1.1% [0.8-1.6] in

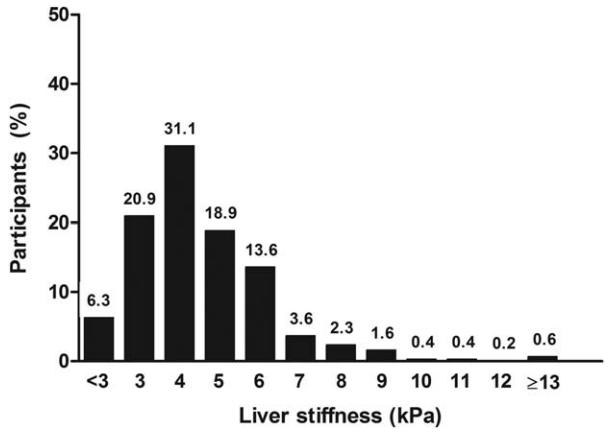

Fig. 2. Distribution of reliable liver stiffness measurements in 3,041 participants.

participants ages 50-60 years vs. 10.5% [6.6-14.6] in participants >80 years; $P = 4.0*10^{-162}$). Probabilities also increased with increasing age for participants with steatosis, but without DM (n = 853; 5.1% [3.6-7.0] vs. 8.8% [5.0-11.0]; $P = 0.001$). For participants with only DM (n = 118), probabilities did not increase with age ($P = 0.76$). Participants with both DM and steatosis had the highest probabilities of having LSM ≥8.0 kPa for all age decades. For these 216 participants, the overall probability of LSM ≥8.0 kPa was 17.2% (12.5-23.4). These probabilities did not increase with age (14.3 [12.1-18.4] vs. 17.0 [12.3-24.1] vs. 20.1 [13.5-23.9] vs. 16.3 [12.5-23.8], respectively, per increasing age decade; overall $P = 0.76$).

We also observed a second interaction in our analyses: The association between current or former smoking and LSM ≥8.0 kPa was significantly influenced by sex (P for interaction term = 0.024). Of 1,902 previously or currently smoking participants, males had a median predicted probability of having LSM ≥8.0 kPa of 6.2% (3.6-10.2), compared to a probability of 3.7% (2.1-6.8) in female smokers ($P < 0.001$). These probabilities were 2.8% (1.6-5.0) and 2.4% (1.3-3.8), respectively, for males and females who had never smoked ($P < 0.001$).

Table 2. Factors Associated With (Log-Transformed) LSM in Linear Multivariable Regression Analysis (n = 3,041)

Variable	β (95% CI)	P Value
Age, per 10 years	0.029 (0.022-0.036)	<0.001
Sex, male	0.055 (0.042-0.067)	<0.001
DM	0.041 (0.023-0.059)	<0.001
Spleen size, cm	0.012 (0.007-0.016)	<0.001
ALT, per 10 U/L	0.014 (0.009-0.018)	<0.001
BMI, kg/m^2	−0.001 (−0.002-0.001)	0.28
Steatosis	0.024 (0.012-0.037)	<0.001
Alcohol consumption, drinks/week	<0.001 (−0.001-0.001)	0.50
Current or former smoking	0.002 (−0.009-0.013)	0.74
HBsAg or anti-HCV positive	0.090 (0.025-0.156)	0.007

Table 3. Factors Associated With LSM ≥8.0 kPa (n = 169) in Logistic Multivariable Regression Analysis

Variable	OR (95% CI)	P Value
Age for no DM, no steatosis, per 10 years	2.40 (1.72-3.36)	<0.001
Sex, male	1.35 (0.91-2.02)	0.14
Spleen size, cm	1.23 (1.09-1.40)	0.001
ALT, per 10 U/L	1.24 (1.12-1.38)	<0.001
BMI, kg/m^2	1.00 (0.95-1.06)	0.97
Alcohol consumption, drinks/week	1.00 (0.98-1.03)	0.76
Current or former smoking	1.77 (1.16-2.70)	0.008
HBsAg or anti-HCV positive	5.38 (1.60-18.0)	0.006
Interaction		
For age 66.0 years*		
No DM, no steatosis	1 (ref)	ref
Steatosis, no DM	1.99 (1.28-3.10)	0.002
DM, no steatosis	3.22 (1.39-7.42)	0.006
DM and steatosis	5.20 (3.01-8.98)	<0.001

*66.0 years was the mean age in the total population.
Abbreviation: ref, reference.

Liver Stiffness in Participants With NAFLD. A subanalysis was performed to investigate factors associated with LSM in participants with NAFLD. Of 3,041 participants, 532 participants were excluded because of presence of secondary causes associated with steatosis (excessive alcohol consumption [n = 449], positive anti-HCV [n = 16], positive HBsAg [n = 7], and pharmacological agents associated with steatosis [n = 69]). Eight hundred twenty-two (32.8%) participants had NAFLD. Mean age of participants with NAFLD was 66.0 ± 7.2 years, mean BMI was 29.8 ± 4.0 kg/m^2, and median LSM was 4.9 kPa (4.0-6.1). Sixty-nine (8.4%) participants of this subgroup had LSM ≥8.0 kPa. In

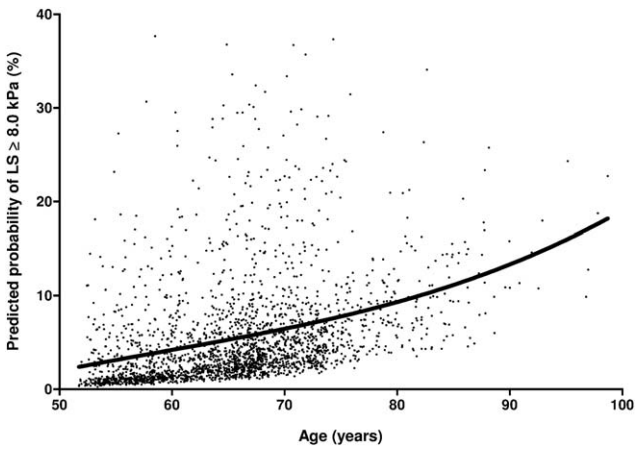

Fig. 3. Predicted probabilities (%) of having LSM ≥8.0 kPa increase with age. Probabilities are adjusted for age, sex, BMI, current or former smoking, presence of DM and/or steatosis, ALT, presence of HBsAg and/or anti-HCV, weekly alcohol consumption, spleen size, and the interaction between age and presence of DM and/or steatosis.

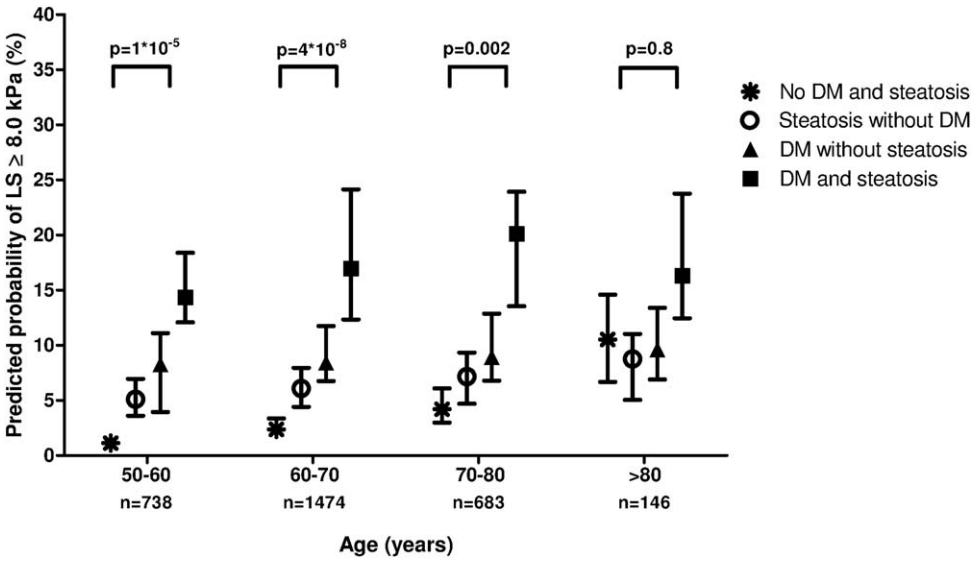

Fig. 4. Predicted probabilities (%) of having LSM ≥8.0 kPa per age decade for participants with and without DM and/or steatosis. Probabilities are adjusted for age, sex, BMI, current or former smoking, presence of DM and/or steatosis, ALT, presence of HBsAg and/or anti-HCV, weekly alcohol consumption, spleen size, and the interaction between age and presence of DM and/or steatosis.

multivariable linear regression analysis, higher age ($\beta = 0.027$ per 10 years; 95% CI: 0.012-0.042; $P < 0.001$), presence of DM ($\beta = 0.038$; 95% CI: 0.010-0.065; $P = 0.007$), male sex ($\beta = 0.044$; 95% CI: 0.019-0.068; $P = 0.001$), higher ALT ($\beta = 0.023$ per 10 U/L; 95% CI: 0.014-0.032; $P < 0.001$), and larger spleen size ($\beta = 0.014$; 95% CI: 0.006-0.023; $P = 0.001$) were associated with higher log LSM. Replacing presence of DM by HOMA-IR did not alter results. Presence of DM (OR, 2.96; 95% CI: 1.60-5.47; $P = 0.001$), higher ALT (OR, 1.43 per 10 U/L; 95% CI: 1.19-1.72; $P < 0.001$), and increasing weekly alcohol consumption (OR, 1.09; 95% CI: 1.01-1.18; $P = 0.023$) were associated with having LSM ≥8.0 kPa in participants with NAFLD in multivariable logistic regression analysis.

Discussion

To date, this is the largest study to investigate factors associated with and distribution of liver fibrosis, assessed by TE, in a general Caucasian population of older individuals. In this cross-sectional study, the prevalence of LSM ≥8.0 kPa, suggestive of clinically relevant liver fibrosis, was 5.6%. Higher age, presence of DM and/or steatosis, higher ALT, larger spleen size, current or former smoking, and positive viral serology for hepatitis B and/or C were independently associated with LSM ≥8.0 kPa.

Only few community-based studies have assessed liver fibrosis in adults by means of TE. In a study by Roulot et al., in which reliable LSM was obtained in 1,190 subjects older than 45 years attending a free medical checkup, prevalence of LSM ≥8.0 kPa was 7.5% and risk factors of LSM ≥8.0 kPa were rather consistent with our findings and included age, elevated liver enzymes, and factors associated with MetS.[21] We were able to include ultrasonographic findings in our analyses and found that both steatosis and spleen size were significantly associated with greater liver stiffness. Although the prevalence of LSM ≥8.0 kPa in our cohorts is nearly comparable, our study population may have included a less-selected population, but had a higher mean age, greater prevalence of MetS, and included more women than the population described by Roulot et al. A cross-sectional, community-based study by Wong et al. assessed the prevalence of NAFLD and LSM ≥9.6 kPa in Hong Kong Chinese subjects by using magnetic resonance imaging and TE.[20] LSM ≥9.6 kPa was present in 2.0% of the total cohort of 759 subjects without a hepatitis B or C infection and in 3.7% of subjects with NAFLD. These percentages were comparable with our results (data not shown), even though the prevalence of steatosis and MetS and the mean age of our cohort were higher.

In the present study, higher age was associated with continuous LSM or elevated LSM in both linear and logistic regression analysis, respectively. An association of age with LSM has been corroborated in some previous studies, but not in others.[21,28-32] Indeed, incidence of liver diseases increases with advancing age, which may, in part, explain a higher prevalence of LSM ≥8.0 kPa at higher age.[33] However, we demonstrated that higher age remained associated with higher LSM after exclusion of subjects with positive viral serology for hepatitis B and/or C, steatosis, ALT above the ULN, and LSM ≥8.0 kPa. Elastic properties of the normal liver may change as a result of aging for several reasons. First, age-related changes in histological architecture of the

liver may cause increased liver stiffness. With aging, there is a decline in hepatic blood flow, hepatic volume, and number and volume of individual hepatocytes.[34,35] Hepatocytes in aged livers show increased polyploidy.[36] Second, livers of older individuals may be stiffer as a result of accumulation of collagen. It is hypothesized that older individuals have reduced collagenolytic activity.[37] In addition, cellular senescence, caused by telomere dysfunction, and increased mitochondrial damage and oxidative stress, may increase susceptibility of the older liver to liver damage and may reduce the capacity of the liver to regenerate.[33]

A compelling observation in our study was that the association of age with LSM ≥8.0 kPa was positively influenced by presence of steatosis and/or DM. Predicted probabilities of LSM ≥8.0 kPa increased with age for participants without DM. For participants with DM, on the other hand, the probability of having clinically relevant fibrosis was already as high as 8.2%-14.0% for participants ages 50-60 years, depending on the concurrent presence of steatosis, and these probabilities did not increase with age. These findings underline the significant role of these—potentially modifiable—risk factors in liver fibrosis and stress the importance of early targeting insulin resistance and/or DM. We observed that the presence of steatosis and/or DM did not influence the probability of LSM ≥8.0 kPa in octo- and nonagenarians, which can be the result of a survival effect, but it may also suggest that both steatosis and insulin resistance or DM play a less important role in liver fibrosis at very old age. However, this remains speculative given that some selection bias may exist. In addition, this study was cross-sectional by design, and we were therefore unable to determine the role of duration of presence of DM or steatosis on probability of high LSM in the very old.

Furthermore, a strong association between increased liver stiffness and presence of DM and/or greater insulin resistance was not only observed in the study population as a whole, but also in a subgroup of subjects with ultrasonographically defined NAFLD. Prevalence of LSM ≥8.0 kPa in participants with NAFLD was as high as 8.4%. These findings suggest that NAFLD may be an important determinant of clinically relevant fibrosis in a population that has a very low prevalence of viral hepatitis. Given that NAFLD will become more prevalent considering population aging and the current increasing prevalence of DM and obesity, and because the presence of NASH is associated with an increased overall, cardiovascular, and liver-related mortality, NAFLD will become a more urgent public health issue in the next few decades.[38-41]

In contrary to the observations by Roulot et al, we found that previous or current smoking was significantly associated with elevated liver stiffness.[21] Smoking may cause liver injury through various mechanisms, including (in)direct toxic effects, immunological effects through proinflammatory cytokine release, and oncogenic effects through free radical damage.[42] The harmful effects of smoking on the liver have been investigated in some experimental studies with rats and in smaller clinical studies including patients with various liver diseases; however, epidemiological evidence of an independent association is limited.[43-50] Although a synergism between alcohol consumption and smoking is well known, smoking was shown to increase the risk of LC independent of alcohol consumption in a previous population-based cohort study, consistent with our study.[45]

The strengths of this study encompass the large number of participants that were included and extensive data that were available for characterization of metabolic and environmental traits. In addition, abdominal US was performed in all participants. Our study also has some limitations. For diagnosing liver fibrosis, biopsy remains the gold standard. However, performing a biopsy in all participants of this population-based cohort would have been unethical. Therefore, we used TE to obtain LSM for all participants. Although limited research is available addressing the PPV to rule in advanced fibrosis and cirrhosis in subjects of the general population, TE is considered to be a reproducible, effective noninvasive alternative to assess liver fibrosis in patients with CLDs.[25,51] In addition, TE has also been shown to be able to predict portal hypertension–related complications, decompensation, and survival in patients with CLD.[52,53] There is some debate regarding which criteria to apply to determine reliability of TE. Applying the other reliability criteria—success rate ≥60%, ≥10 valid measurements, and IQR/median LSM ≤0.3—to our cohort resulted in comparable results. Unfortunately, we did not have access to histology to corroborate our results. However, we expect the cutoff of 8.0 kPa for clinically relevant fibrosis to be feasible, given that Roulot et al. could determine a cause of liver disease in all subjects with LSM ≥8.0 kPa in whom a biopsy was performed.[21] XL-probe may have a lower cut-off value for advanced liver fibrosis, and different cutoffs have been applied for advanced fibrosis in patients with CLDs depending on etiology.[15,25] Consistency of results was found when different cutoffs were used in our study. Steatosis was diagnosed by means of USG, which may identify a degree of steatosis greater than 30%. Nevertheless, abdominal USG has an acceptable sensitivity of

80%-100% for detecting steatosis, and its accuracy for diagnosis of steatosis meets other imaging modalities.[54-56] Finally, there are controversial data about the influence of liver steatosis on LSM values in chronic hepatitis C patients.[57-60] In patients with NAFLD, most studies have shown no, or an inverse, relationship between degree of steatosis and LSM.[16,19,61] Petta et al. demonstrated an association between severity of steatosis and LSM in NAFLD patients without significant fibrosis at biopsy. In this study, a cutoff of 7.9 kPa—similar to the cutoff used in the present study—was identified as the best LSM cutoff for discriminating significant fibrosis in presence of severe steatosis (≥66%) or severe bright liver echo pattern.[62] We demonstrated no association between severity of steatosis and elevated liver stiffness in participants with steatosis. However, we cannot completely rule out the possibility that steatosis in itself may have affected LSM, independent of fibrosis stage, owing to the lack of biopsies in this general population.

In summary, prevalence of LSM higher than 8.0 kPa, suggestive of clinically relevant liver fibrosis, was 5.6% in this older adult population-based cohort. Factors associated with clinically relevant fibrosis were higher age, presence of DM and/or steatosis, higher ALT, greater spleen size, current or former smoking, and positive viral serology for hepatitis B and/or C. Presence of DM, especially in concurrent presence of steatosis, resulted in increased probabilities of having clinically relevant fibrosis. In the context of an aging population and increasing prevalence of obesity, DM, and NAFLD, this study illustrates that liver fibrosis and its complications may become a more prominent public health issue in the near future.

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References

1. World Health Organization. Global health observatory data repository. Causes of death 2002-2012. http://apps.who.int/gho/data/node.main.887?lang=en. Accessed August 27, 2015.
2. Lim YS, Kim WR. The global impact of hepatic fibrosis and end-stage liver disease. Clin Liver Dis 2008;12:733-746, vii.
3. Heron M. Deaths: leading causes for 2010. Natl Vital Stat Rep 2013;62:1-96.
4. Blachier M, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. J Hepatol 2013;58:593-608.
5. Bedogni G, Miglioli L, Masutti F, Castiglione A, Croce LS, Tiribelli C, Bellentani S. Incidence and natural course of fatty liver in the general population: the Dionysos study. Hepatology 2007;46:1387-1391.
6. Caballeria L, Pera G, Auladell MA, Toran P, Munoz L, Miranda D, et al. Prevalence and factors associated with the presence of nonalcoholic fatty liver disease in an adult population in Spain. Eur J Gastroenterol Hepatol 2010;22:24-32.
7. Haring R, Wallaschofski H, Nauck M, Dorr M, Baumeister SE, Volzke H. Ultrasonographic hepatic steatosis increases prediction of mortality risk from elevated serum gamma-glutamyl transpeptidase levels. Hepatology 2009;50:1403-1411.
8. Koehler EM, Schouten JN, Hansen BE, van Rooij FJ, Hofman A, Stricker BH, Janssen HL. Prevalence and risk factors of non-alcoholic fatty liver disease in the elderly: results from the Rotterdam study. J Hepatol 2012;57:1305-1311.
9. Williamson RM, Price JF, Glancy S, Perry E, Nee LD, Hayes PC, et al. Prevalence of and risk factors for hepatic steatosis and nonalcoholic fatty liver disease in people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. Diabetes Care 2011;34:1139-1144.
10. Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care 2007;30:1212-1218.
11. Floreani A. Liver diseases in the elderly: an update. Dig Dis 2007;25:138-143.
12. From the Centers for Disease Control and Prevention. Public health and aging: trends in aging—United States and worldwide. JAMA 2003;289:1371-1373.
13. Frith J, Jones D, Newton JL. Chronic liver disease in an ageing population. Age Ageing 2009;38:11-18.
14. Castera L. Noninvasive methods to assess liver disease in patients with hepatitis B or C. Gastroenterology 2012;142:1293-1302.e4.
15. Myers RP, Pomier-Layrargues G, Kirsch R, Pollett A, Duarte-Rojo A, Wong D, et al. Feasibility and diagnostic performance of the FibroScan XL probe for liver stiffness measurement in overweight and obese patients. Hepatology 2012;55:199-208.
16. Wong VW, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology 2010;51:454-462.
17. Verveer C, Zondervan PE, ten Kate FJ, Hansen BE, Janssen HL, de Knegt RJ. Evaluation of transient elastography for fibrosis assessment compared with large biopsies in chronic hepatitis B and C. Liver Int 2012;32:622-628.
18. Friedrich-Rust M, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology 2008;134:960-974.
19. **Yoneda M**, **Yoneda M**, Mawatari H, Fujita K, Endo H, Iida H, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). Dig Liver Dis 2008;40:371-378.
20. Wong VW, Chu WC, Wong GL, Chan RS, Chim AM, Ong A, et al. Prevalence of non-alcoholic fatty liver disease and advanced fibrosis in Hong Kong Chinese: a population study using proton-magnetic resonance spectroscopy and transient elastography. Gut 2012;61:409-415.
21. Roulot D, Costes JL, Buyck JF, Warzocha U, Gambier N, Czernichow S, et al. Transient elastography as a screening tool for liver fibrosis and cirrhosis in a community-based population aged over 45 years. Gut 2011;60:977-984.
22. Hofman A, Darwish Murad S, van Duijn CM, Franco OH, Goedegebure A, Ikram MA, et al. The Rotterdam Study: 2014 objectives and design update. Eur J Epidemiol 2013;28:889-926.
23. Hamaguchi M, Kojima T, Itoh Y, Harano Y, Fujii K, Nakajima T, et al. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. Am J Gastroenterol 2007;102:2708-2715.
24. Boursier J, Zarski JP, de Ledinghen V, Rousselet MC, Sturm N, Lebail B, et al. Determination of reliability criteria for liver stiffness evaluation by transient elastography. Hepatology 2013;57:1182-1191.

25. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol 2008;48:835-847.
26. **Grundy SM**, **Cleeman JI**, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome—an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation 2005;112:2735-2752.
27. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-419.
28. Roulot D, Czernichow S, Le Clesiau H, Costes JL, Vergnaud AC, Beaugrand M. Liver stiffness values in apparently healthy subjects: influence of gender and metabolic syndrome. J Hepatol 2008;48:606-613.
29. Colombo S, Belloli L, Zaccanelli M, Badia E, Jamoletti C, Buonocore M, Del Poggio P. Normal liver stiffness and its determinants in healthy blood donors. Dig Liver Dis 2011;43:231-236.
30. Salles N, Dussarat P, Foucher J, Villars S, de Ledinghen V. Non-invasive evaluation of liver fibrosis by transient elastography and biochemical markers in elderly inpatients. Gastroenterol Clin Biol 2009;33:126-132.
31. Das K, Sarkar R, Ahmed SM, Mridha AR, Mukherjee PS, Dhali GK, et al. "Normal" liver stiffness measure (LSM) values are higher in both lean and obese individuals: a population-based study from a developing country. Hepatology 2012;55:584-593.
32. Sirli R, Sporea I, Tudora A, Deleanu A, Popescu A. Transient elastographic evaluation of subjects without known hepatic pathology: does age change the liver stiffness? J Gastrointestin Liver Dis 2009;18:57-60.
33. Hoare M, Das T, Alexander G. Ageing, telomeres, senescence, and liver injury. J Hepatol 2010;53:950-961.
34. Wynne HA, James OF. The ageing liver. Age Ageing 1990;19:1-3.
35. Schmucker DL, Sanchez H. Liver regeneration and aging: a current perspective. Curr Gerontol Geriatr Res 2011;2011:526379.
36. Schmucker DL. Hepatocyte fine structure during maturation and senescence. J Electron Microsc Tech 1990;14:106-125.
37. Gagliano N, Arosio B, Grizzi F, Masson S, Tagliabue J, Dioguardi N, et al. Reduced collagenolytic activity of matrix metalloproteinases and development of liver fibrosis in the aging rat. Mech Ageing Dev 2002; 123:413-425.
38. Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005;129:113-121.
39. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006;44:865-873.
40. Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. J Hepatol 2008; 49:608-612.
41. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N Engl J Med 2010;363: 1341-1350.
42. El-Zayadi AR. Heavy smoking and liver. World J Gastroenterol 2006; 12:6098-6101.
43. **Azzalini L**, **Ferrer E**, Ramalho LN, Moreno M, Dominguez M, Colmenero J, et al. Cigarette smoking exacerbates nonalcoholic fatty liver disease in obese rats. Hepatology 2010;51:1567-1576.
44. Bolukbas FF, Kabasakal L, Bolukbas C, Uysal MK, Peker O, Ovunc O. Hepatocyte damage caused by nicotine alone and in combination with alcohol. Gastroenterology 1998;114:A1318-A1319.
45. Dam MK, Flensborg-Madsen T, Eliasen M, Becker U, Tolstrup JS. Smoking and risk of liver cirrhosis: a population-based cohort study. Scand J Gastroenterol 2013;48:585-591.
46. Dev A, Patel K, Conrad A, Blatt LM, McHutchison JG. Relationship of smoking and fibrosis in patients with chronic hepatitis C. Clin Gastroenterol Hepatol 2006;4:797-801.
47. Hezode C, Lonjon I, Roudot-Thoraval F, Mavier JP, Pawlotsky JM, Zafrani ES, Dhumeaux D. Impact of smoking on histological liver lesions in chronic hepatitis C. Gut 2003;52:126-129.
48. Pessione F, Ramond MJ, Njapoum C, Duchatelle V, Degott C, Erlinger S, et al. Cigarette smoking and hepatic lesions in patients with chronic hepatitis C. Hepatology 2001;34:121-125.
49. Corrao G, Lepore AR, Torchio P, Valenti M, Galatola G, Damicis A, et al. The effect of drinking coffee and smoking cigarettes on the risk of cirrhosis associated with alcohol-consumption—a case-control study. Eur J Epidemiol 1994;10:657-664.
50. Yu MW, Hsu FC, Sheen IS, Chu CM, Lin DY, Chen CJ, Liaw YF. Prospective study of hepatocellular carcinoma and liver cirrhosis in asymptomatic chronic hepatitis B virus carriers. Am J Epidemiol 1997; 145:1039-1047.
51. Rockey DC. Noninvasive assessment of liver fibrosis and portal hypertension with transient elastography. Gastroenterology 2008;134:8-14.
52. **Robic MA**, **Procopet B**, Metivier S, Peron JM, Selves J, Vinel JP, Bureau C. Liver stiffness accurately predicts portal hypertension related complications in patients with chronic liver disease: a prospective study. J Hepatol 2011;55:1017-1024.
53. Vergniol J, Foucher J, Terrebonne E, Bernard PH, le Bail B, Merrouche W, et al. Noninvasive tests for fibrosis and liver stiffness predict 5-year outcomes of patients with chronic hepatitis C. Gastroenterology 2011;140:1970-U1197.
54. Adams LA, Talwalkar JA. Diagnostic evaluation of nonalcoholic fatty liver disease. J Clin Gastroenterol 2006;40(Suppl 1):S34-S38.
55. Wieckowska A, McCullough AJ, Feldstein AE. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: present and future. Hepatology 2007;46:582-589.
56. **Hernaez R**, **Lazo M**, Bonekamp S, Kamel I, Brancati FL, Guallar E, Clark JM. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 2011;54:1082-1090.
57. Boursier J, de Ledinghen V, Sturm N, Amrani L, Bacq Y, Sandrini J, et al. Precise evaluation of liver histology by computerized morphometry shows that steatosis influences liver stiffness measured by transient elastography in chronic hepatitis C. J Gastroenterol 2014;49:527-537.
58. Lupsor M, Badea R, Stefanescu H, Grigorescu M, Sparchez Z, Serban A, et al. Analysis of histopathological changes that influence liver stiffness in chronic hepatitis C. Results from a cohort of 324 patients. J Gastrointestin Liver Dis 2008;17:155-163.
59. Macaluso FS, Maida M, Camma C, Cabibbo G, Cabibi D, Alduino R, et al. Steatosis affects the performance of liver stiffness measurement for fibrosis assessment in patients with genotype 1 chronic hepatitis C. J Hepatol 2014;61:523-529.
60. Sanchez-Conde M, Montes Ramirez ML, Bellon Cano JM, Caminoa A, Alvarez Rodriguez F, Gonzalez Garcia J, et al. Impact of liver steatosis on the correlation between liver stiffness and fibrosis measured by transient elastography in patients coinfected with human immunodeficiency virus and hepatitis C virus. J Viral Hepat 2011;18:e278-e283.
61. Gaia S, Carenzi S, Barilli AL, Bugianesi E, Smedile A, Brunello F, et al. Reliability of transient elastography for the detection of fibrosis in non-alcoholic fatty liver disease and chronic viral hepatitis. J Hepatol 2011;54:64-71.
62. Petta S, Maida M, Macaluso FS, Marco VD, Cammà C, Cabibi D, Craxì A. The severity of steatosis influences liver stiffness measurement in patients with nonalcoholic fatty liver disease. Hepatology 2015 Apr 9. doi: 10.1002/hep.27844. [Epub ahead of print]

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